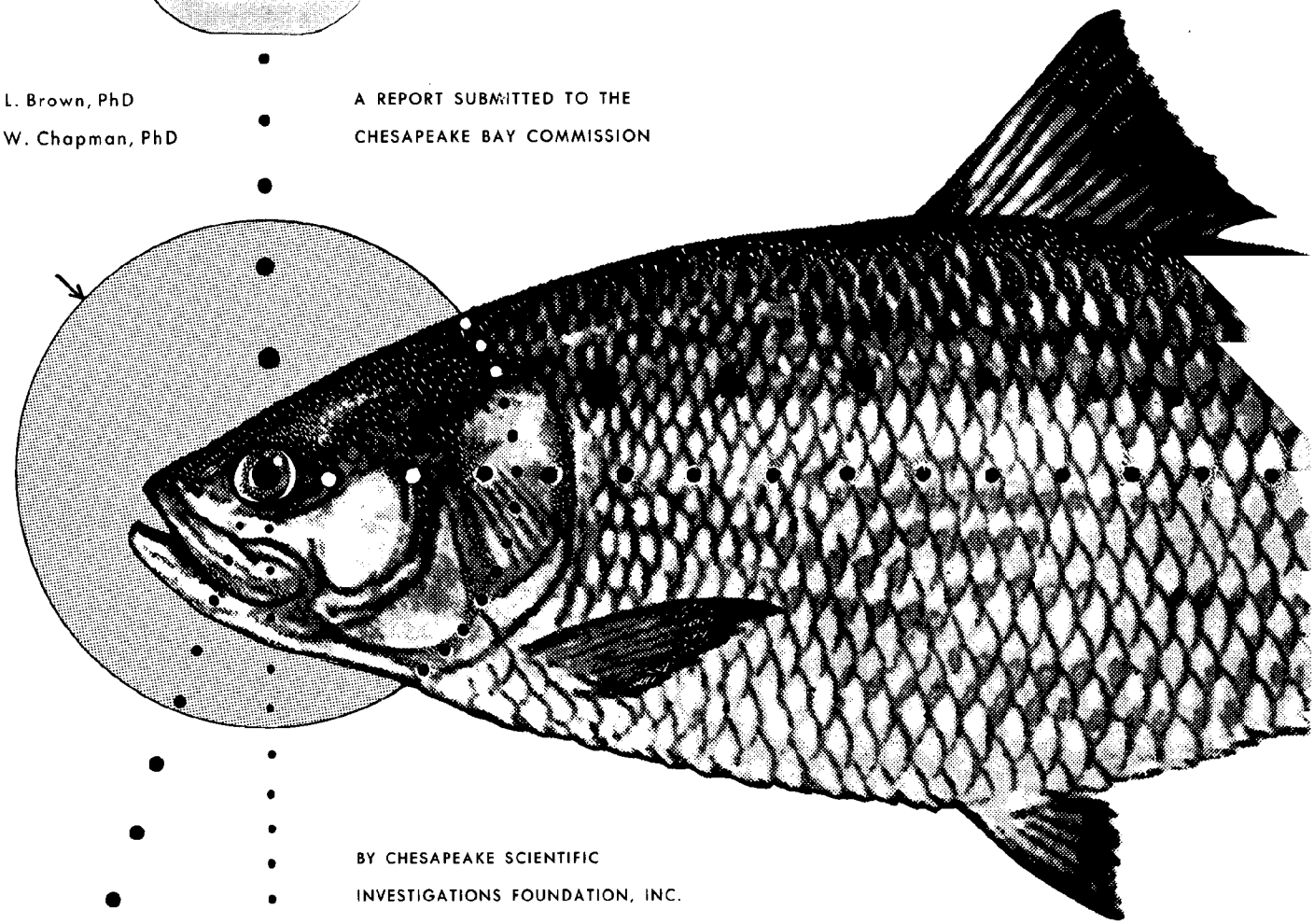
GENETIC ANALYSIS OF AMERICAN SHAD ENTERING CHESAPEAKE BAY

Bonnie L. Brown, PhD
Robert W. Chapman, PhD

A REPORT SUBMITTED TO THE
CHESAPEAKE BAY COMMISSION



BY CHESAPEAKE SCIENTIFIC
INVESTIGATIONS FOUNDATION, INC.

SEPTEMBER, 1991

GENETIC ANALYSIS OF AMERICAN SHAD ENTERING CHESAPEAKE BAY

Principal Investigators:

Bonnie L. Brown, Ph.D.
Department of Biology
Virginia Commonwealth University
Richmond, VA 23284-2012

Robert W. Chapman, Ph.D.
Department of Biology
East Carolina University
Greenville, NC 27858-4353

A report submitted to:

The Chesapeake Bay Commission
September, 1991

By:

Chesapeake Scientific Investigations Foundation, Inc.

This contracted research was funded by the Pennsylvania Delegation to the Chesapeake Bay Commission, the Virginia Council on the Environment (COE) and the Virginia Marine Resources Commission. COE funding was provided through the Coastal Resources Management Program through grant # NA90AA-H-CZ796 of the National Oceanic and Atmospheric Administration under the Coastal Zone Management Act of 1972 as amended.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This study was conducted for the Chesapeake Bay Commission. Funds were provided by the Chesapeake Bay Commission, the Virginia Council on the Environment (Coastal Zone Management Grant # NA90AA-H-CZ796), and the Virginia Marine Resources Commission (in-kind services) to perform genetic analysis of American shad, *Alosa sapidissima*, harvested by Virginia watermen from waters outside Chesapeake Bay in Virginia's ocean gill net fishery. Genetic analysis was employed to ascertain, to the maximum extent possible, the natal stream and spawning destination of shad which are susceptible to harvest in the Atlantic Ocean intercept fishery. Genotypes were compared to shad from ten baseline target rivers and from Susquehanna River previously analyzed under MDNR contract number PR90-004-004. Inclusion of Albemarle Sound, Cape Fear and Santee River baseline populations was made possible by a supplemental grant from Atlantic States Marine Fisheries Commission's Interstate Fisheries Management Program (ISFMP) grant number 91-1(PASRH). We thank J. Pella for editing an earlier version of this report and for providing the GIRLSEM algorithm which we used to analyze the genetic data produced by this study. We also thank Barney Jernigan for preparing other essential computer programs and for his assistance with data analysis. A great many other people also contributed to the study by providing biological specimens of American shad including but not limited to:

Walt Ambrogetti, USFWS
Tom Backman, USFWS
Bob Brandt, NY State Dept. of Environmental Conservation
Rick Eager, USFWS
Chris Frese, RMC Environmental Services
Joe Loesch, Virginia Inst. of Marine Science
Art Lupine, NJ Freshwater Fisheries Lab
Ted Meyers, USFWS
Roy Miller, DE Div. of Fisheries & Wildlife
Robert O'Reilly, Virginia Marine Resources Commission
Jim Owens, Virginia Inst. of Marine Science
Fritz Rhode, NC Div. of Marine Fisheries
Dick St. Pierre, USFWS
Mark Tisa, MA Div. Fisheries & Wildlife
Glenn Ulrich, SC Wildlife & Marine Resources Div.
Dale Weinrich, MD Dept. of Natural Resources
Sara Winslow, NC Div. of Marine Fisheries

EXECUTIVE SUMMARY

Genetic analysis was employed to examine the stock composition of American shad, *Alosa sapidissima*, harvested outside Chesapeake Bay in Virginia's Atlantic Ocean intercept fishery during the Spring of 1991. Genotypes of intercept fish were compared to fish from thirteen American shad populations in order to estimate the relative percentage of shad from each baseline population in the intercept sample. Techniques involved restriction endonuclease digestion of mitochondrial DNA (mtDNA) purified from shad egg tissue, a common methodology used for examining population dynamics in fishes. MtDNA genotypes were obtained for 158 individuals from three locations near the mouth of Chesapeake Bay: Rudee Inlet, Chincoteague, and Quinby, VA. The thirteen baseline populations included 549 American shad from spawning aggregations in ten target rivers (Connecticut, Delaware, Hudson, Nanticoke, Pamunkey, James, Chowan, Savannah, St. Johns and Santee Rivers), from the Susquehanna Flats in upper Chesapeake Bay, and from Susquehanna River shad lifted over Conowingo Dam in 1990 and 1991.

Yearly, since 1982, the Susquehanna has been stocked with shad from several of the following rivers: Columbia, Connecticut, Delaware, Hudson, James and Pamunkey. Genetic analysis of Susquehanna shad in 1990 and 1991 indicated that the Susquehanna shad population is now a mixed-stock composed of native Susquehanna, native Chesapeake Bay, Connecticut, Hudson, Pamunkey and Santee lineages. Therefore, in the genetic analysis of the Atlantic Ocean fishery, occurrence of those lineages which have been stocked into the Susquehanna is taken to indicate the presence of Susquehanna fish.

Since many shad have mtDNA genotypes which are common to several if not all drainages, every fish cannot be uniquely identified to a river system. Therefore, when a mixed assemblage of fish is examined (such as an ocean fishery), genotypes of the entire group are statistically analyzed for comparison to baseline genetic data for potential source populations. The analysis provides an estimate of the most likely composition of the group in question. We have employed a standard statistical treatment, maximum likelihood analysis, which was modified by Dr. J. Pella and co-workers to interpret mixed-fishery genetic data. This analysis has been successfully employed to manage the west coast salmon fishery for many years.

Most baseline American shad populations share one common mtDNA type, but each contains unique types as well. These unique types of mtDNA made it possible to estimate the percentage of each baseline stock represented in the migrating coastal group of shad. Nineteen percent of American shad sampled outside Chesapeake Bay had unique mtDNAs themselves and could not be classified as originating from any of our current baseline populations. The remaining 81% of intercept shad were compared to the baseline populations in a maximum likelihood analysis of stock contribution. Composition of this portion of the intercept group was estimated to be due to contributions of the following seven stocks: St. Johns (2 ± 2 %), Pamunkey (2 ± 2 %),

Chowan (5 ± 5 %), Santee (19 ± 12 %), Hudson (9 ± 5 %), Susquehanna (31 ± 10 %) and Connecticut (32 ± 11%). No contribution was detected from the other baseline shad populations.

Given the stocking history and composition of the Susquehanna River population as shown in Tables 1 and 5, it is possible that portions of the estimated contributions for some baseline stocks, particularly Santee River, are actually due to the presence of Susquehanna shad in the intercept sample. For example, the genetic analysis does not distinguish individual Pamunkey shad from Pamunkey River from those which were stocked into Susquehanna River. Statistical analysis of the genetic data employs frequencies of Pamunkey genotypes as they occur in both the Pamunkey and Susquehanna to estimate the most likely relative contribution of each of the two stocks. Therefore, in the absence of compelling information such as tag returns or additional genetic data, the estimated contribution to the intercept fishery of river stocks previously introduced into the Susquehanna are taken as the actual preliminary estimates of those rivers' contributions to the intercept fishery. This conservative approach avoids underestimating contributions by those stocks. However, in the case of Santee River we do have compelling evidence to support the assumption that the contribution estimated for Santee River (originally included in the analysis as an outgroup) is due at least in part to the presence of Susquehanna shad in the intercept samples. First, Jesien and Hocutt (1991) report that for shad tagged off Rudee Inlet, 28% of tag returns were from north of Chesapeake Bay and only 17% of returns came from areas south of the tagging location (all from North Carolina). The remining 55% of shad tag returns were from fish which moved into Chesapeake Bay. This information, combined with knowledge of genetic similarity between the Susquehanna and Santee stocks (Chapman and Brown, 1991), knowledge of Santee River shad migration and spawning (G. Ulrich, pers. communication), and the geographic separation of Santee stocks from the intercept fishery, indicates that it is appropriate to group the Susquehanna and Santee contribution estimates (Wood *et al.* 1987). Thus, we estimate that Virginia's 1991 intercept fishery was comprised of at least 41 % Susquehanna shad (0.81 x (31_{Sus} + 19_{Santee} %)).

Despite small sample sizes, a trend is evident in the genetic composition of shad harvested north of the Bay mouth (Chincoteague and Quinby) and those harvested south of the Bay mouth (Rudee). Chi-square analysis indicates that the stock compositions of shad harvested from the two regions are significantly different. In fact, a large portion of Rudee Inlet shad were of Virginia origin while the Chincoteague and Quinby harvests were composed primarily of Susquehanna, Hudson and Santee lineages.

The estimates provided in this report should be considered preliminary for four important reasons. First, several of the baseline samples are very small and may not adequately represent those groups of American shad. Second, contributions attributed by genetic analysis to Santee and some other rivers may be due in part to the presence of Susquehanna shad which appear to be largely of Connecticut, Nanticoke and Santee River descent. Third, since the Susquehanna River population has not attained genetic stability, it is possible that contribution estimates for this river

will vary from year to year as the resurgent Susquehanna population approaches a stable genetic equilibrium causing the estimated composition of the intercept fishery to vary. Finally, the fact that 19% of intercept shad were distinguished by unique genotypes indicates that either the existing baseline populations have been inadequately characterized or that one or more potential source populations have not been included in the baseline data set. These significant points are discussed in the report and lead to recommendations for procedural refinements which must be incorporated into future American shad research.

To consider the potential effect of the coastal intercept fishery one would need several years of estimates of the stock composition and magnitude of both Maryland and Virginia's coastal harvests. However, a rough estimate can be made based on Virginia's preliminary estimate of their 1991 ocean shad harvest and the present genetic data. The estimated amount of intercept shad harvested in 1991 was 405,612 pounds (at 4.5 pounds per fish, this is 90,136 shad). Multiplying by the factor of 0.41, approximately 36,955 intercepted shad were of Susquehanna origin. This value exceeds the number of shad lifted over Conowingo Dam in the Spring of 1991. If these findings are verified by future monitoring, tagging and genetic evaluation then the most conservative action would be to restrict shad harvests along the Atlantic coast.

INTRODUCTION

The American shad, *Alosa sapidissima*, is an anadromous member of the herring family (Clupeidae), which ranges from the Gulf of St. Lawrence to Florida (Walburg and Nichols, 1967). During its springtime spawning runs the species has been subjected to substantial commercial and recreational fishing pressure throughout its range, particularly in Chesapeake Bay tributaries and by Maryland and Virginia's ocean fisheries. In addition, shad populations of almost every Chesapeake Bay drainage have been further restricted by dams which block migration to their spawning habitat in fresh water transition zones. As a result of fishery exploitation, loss of spawning and nursery habitat, and possibly environmental degradation such as stream acidification, harvests of shad in Pennsylvania, Maryland, the District of Columbia and Virginia declined precipitously during the period 1965-1988 (Stagg, 1986; Gibson, Crecco and Stang, 1988).

A great deal of effort has been expended to revive stocks of Chesapeake Bay American shad. Conservation and restoration measures were enacted in Pennsylvania, Maryland and the District of Columbia in the early 1980s. Some agencies required season, gear and by-catch restrictions along with creel limits to reduce fishing effort (Maryland's shad fishery was closed in 1980). Concurrently, the issues of habitat loss and degradation were addressed by installing permanent fish passage facilities such as the one at Conowingo Dam, removing some obstructions to migrating fish, re-stocking fish into historical spawning habitats, establishing stock assessment and monitoring programs, and operating dam turbines in a manner which maintained minimum flow and standard dissolved oxygen levels (CEC, 1989).

Prior to these efforts, American shad migrating upstream in Susquehanna River had been few in number. Throughout the 1970s shad transported by the trap/lift assembly at Conowingo Dam averaged 127 fish per year (ASMFC, 1988). Yearly release of shad fry and of live pre-spawned adult shad from six other source rivers accompanied the lift operation beginning in 1982 (Table 1). By 1989, more than 6000 migrating shad were reported to have been hauled upstream above all dams to the historical Susquehanna spawning areas.

In view of the apparent success of shad management efforts in Susquehanna River, a program of study was proposed to the Maryland DNR Chesapeake Bay Research and Monitoring Division's Power Plant Topical Research Program designed to examine population dynamics underlying the resurgent American shad population in Susquehanna River using molecular genetic techniques (Chapman and Brown, 1991). Mitochondrial DNA genotypes of American shad being moved over Conowingo Dam were compared to genotypes of shad from the source rivers, from other Chesapeake Bay rivers and from several southern east coast shad populations. Variation in mitochondrial DNA was analyzed and employed to estimate the percent contribution by any of these shad populations to the increasing Susquehanna stock.

Population genetic data collected for the Maryland DNR study were employed in the present study for Chesapeake Bay Commission to estimate the relative percentage of American shad from each baseline population being harvested in Virginia's Atlantic Ocean intercept fishery during the Spring of 1991. The baseline populations available for comparison with the Spring 1991 coastal fishery were: Delaware River, DE (DEL), Hudson River, NY (HUD), Connecticut River, CT (CT), Nanticoke River, MD (NAN), Pamunkey River, VA (PAM), James River, VA (JAM), Chowan River, NC (CHO), Savannah River, SC (SAV), Santee River, SC (SAN), St. Johns River, FL (STJ), the Susquehanna Flats in upper Chesapeake Bay (SF), and Susquehanna River shad lifted over Conowingo Dam in 1990 (S90) and 1991 (S91). This report summarizes the seven-month research project, provides estimates of proportions of American shad from each of the groups outlined above which comprise the Spring 1991 coastal harvest, and outlines management and research implications of these data.

LABORATORY ANALYSIS

The laboratory procedures described in this report are intentionally brief. Detailed instructions for the extraction and digestion of mitochondrial DNA can be found in Chapman and Brown (1990).

During the Spring of 1991, mitochondrial DNA (mtDNA) was extracted from American shad harvested in Virginia's Atlantic Ocean intercept fishery outside the mouth of Chesapeake Bay. On ten occasions during the period 5-27 March, shad were obtained from commercial fishermen landing at three locations: Rudee Inlet, Chincoteague and Quinby/Wachapreague (see Figure 1). Ovaries from each individual fish were removed and placed in a Ziplock Baggie along with an envelope containing a scale sample and pertinent data on the location of capture, size of fish, etc. Each baggie was sealed and placed on wet ice for transportation to the laboratory at East Carolina University in Greenville, NC.

All shad samples arrived at the laboratory on the day they were collected. Within one day of sampling, approximately 10 g of egg tissue from each individual fish were processed to isolate and purify mtDNA. The mtDNA was rehydrated in 150 µl of sterile distilled water and aliquots of 8.5 µl mtDNA from each fish were combined with 0.5 Unit of the following restriction endonucleases (*Aat* I, *Apa* I, *Bcl* I, *Bgl* I, *Dra* I, *Eco*R I, *Eco*R V, *Hin*d III, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, *Sma* I, *Sst* II, and *Xba* I) along with 1 µl of the appropriate buffer supplied by the manufacturer. Each digest was incubated at 37 °C for 3-4 hours and contained a total volume of 10 µl. Reactions were stopped with 1 µl of STOP solution (0.89 M Tris, 0.89 M boric acid, 0.02 M EDTA, 0.25 % bromophenol blue, 50 % glycerol and 1 % SDS) and were electrophoresed overnight through 0.8 % agarose gels. The DNA in gels was stained with ethidium bromide and photographed under ultra-violet light as described by Chapman and Powers (1984).

Restriction digest patterns were recorded for each restriction endonuclease digest of each fish's mtDNA. Digestion patterns were assigned upper-case alphabetic symbols. Then, each individual was assigned a composite "haplotype" consisting of the letters designating the restriction fragment patterns produced by digestion with each of the fifteen enzymes.

DATA ANALYSIS

Details of the mathematical properties of the algorithms used to perform statistical analyses can be found in Sokal and Rohlf (1981), Roff and Bentzen (1989), Fournier *et al.* (1984), Pella (1986), Pella and Milner (1987) and Wood *et al.* (1987).

Each fish's haplotype is a multiple characterization of that fish's mitochondrial genome and is transmitted in a manner analagous to human surnames. As in other animals, variation in shad mtDNA is typified by the occurrence of rare haplotypes in each population (Bentzen *et al.*, 1988 and 1989). If chi-square contingency tests were to be performed the rare haplotypes would be lumped and only the most frequent haplotypes would be employed in the analysis. This practice of pooling rare mtDNA haplotypes results in a severe loss of information relevant to geographic and temporal genetic variation. To resolve this problem, Roff and Bentzen (1989) presented a chi-square analysis which does not require pooling of rare variants. The analysis generates Monte Carlo distributions of expected chi-square from unpooled mtDNA data allowing high levels of significance even when sample sizes are small.

Haplotype frequencies were employed to determine whether baseline samples were distinguishable by the mtDNA genetic analysis and to examine basic genetic relationships between the baseline shad populations and the intercept population. First, chi-square statistics for heterogeneity of mtDNA haplotype frequencies were calculated (Roff and Bentzen, 1989). The chi-square analysis was conducted by initially treating all of the populations as one large assemblage. Based on the finding of significant heterogeneity, successively smaller sets of populations were analyzed until no further heterogeneity was detected. Finally, chi-square was determined between each pair of populations.

The actual contribution of each baseline population to the intercept sample was estimated from mtDNA information by conditional maximum likelihood estimation of stock composition. This approach has been extensively used in population genetics since the early 1930s (Fisher, 1958; Crow and Kimura, 1970) and was reviewed by Pella and Millner (1987). The algorithm we used, called GIRLSEM, was proposed by Fournier *et al.* (1984) and modified by J. Pella of NMFS (Pella, 1986) for interpretation of mixed-fishery genetic data for salmon stocks from California to Alaska. The assumptions of maximum likelihood analysis are: 1) baseline stocks which potentially contribute to the mixture are genetically distinguishable, 2) sampling of baseline stocks is sufficiently precise to identify a significant portion of genetic diversity within each, 3) all source populations represented in the mixture are part of the baseline data set, and 4) a sufficiently large random sample is obtained from the mixed-stock fishery. The accuracy of this program for mtDNA data was tested by analyzing several artificial mixed populations of known composition created by randomly sampling the baseline haplotypes (with replacement) prior to the analysis of mixed shad populations. This accomplished two purposes. First, it tested the general robustness of the program's output with our baseline mtDNA data and, second, it identified populations

whose contributions were consistently over- or under-estimated. Data were entered into the GIRLSEM analysis by treating haplotypes as "multiple alleles at a single locus." The contribution of all 13 baseline populations was then estimated based on the distribution of all 162 haplotypes.

RESULTS

A total of 158 individual shad collected during a one-month period from three locations near the mouth of Chesapeake Bay were processed. Fifteen restriction endonuclease digests were performed per individual for a total of 2,430 digests. Thirty-three restriction fragment profiles were observed in the intercept samples, encompassing seventy-eight separate restriction fragments of the *Alosa sapidissima* mitochondrial genome. Whenever possible the profiles for an enzyme were compared to those obtained by Bentzen *et al.* (1988 and 1989). Restriction fragment profiles for each enzyme are illustrated in Fig. 2.

Genotypes for each individual intercept shad are shown in Appendix A while those for individual baseline shad can be found in Chapman and Brown (1991). Some individuals in both studies were heteroplasmic for mtDNA size and/or site variation. That is, single shad sometimes had mtDNA molecules of different size and/or nucleotide sequence. This phenomenon was previously reported for shad by Bentzen *et al.* (1988 and 1989). In all tables of this report, a single-enzyme genotype denoted as "A/B" indicates the heteroplasmic combination of genotypes A and B in an individual. The same is true for genotypes A/C, A/E, etc. Haplotypes, the composites of all fifteen genotypes, for each intercept shad are shown in Table 2 and are also listed in Appendix B alongside haplotypes for all of the baseline populations. Thirty-two different haplotypes were detected in the intercept samples. Seventy-nine shad had either the common haplotype AAAAAAAAAAAAAAA or one of the many unique baseline haplotypes. Haplotypes of eighteen other individuals in the intercept sample were unique. In some instances the mtDNA preparation was of inadequate quantity or quality to perform all fifteen digests. Sixty-five additional shad were incompletely characterized and were not included in the final analysis (missing data are shown by "-" in Appendix A).

Results of a chi-square analysis based on the distribution of haplotypes are presented in Table 3. The first tier of the chi-square analysis was performed with the entire baseline sample as one assemblage (overall chi-square of 2445.18, $P < 0.0001$) indicating that significant differences existed within the aggregation. The assemblage was successively decomposed by population to the point where no further heterogeneity was detected. In the end, pairwise comparisons between baseline populations were necessary as each baseline sample (except SF) was found to be significantly different from virtually every other baseline sample. Significant differences between baseline samples were taken to indicate that they were distinct genetic stocks. An additional chi-square analysis found that shad harvested from Rudee Inlet were significantly different from those taken at Chincoteague and Quinby (chi-square of 94.78, $P < 0.001$) while no significant difference was detected between the Chincoteague and Quinby samples (chi-square of 19.45, $P = .717$).

Tests for general robustness of the GIRLSEM program were performed on multiple simulated mixed-stock populations created by random selection from the 549 shad genotypes in the baseline data set. Simulated mixture sizes ranged from 52 to 356. Table 4 lists the results of one

such simulation. The simulations indicated that contributions of three baseline populations (Connecticut, Chowan and Delaware) were consistently misrepresented in the synthetic populations. This was expected since while most of the baseline populations surveyed had 18% or more unique individuals, our samples for Chowan and Connecticut Rivers had low proportions of unique individuals. Statistical analysis indicated that Connecticut River was consistently under-represented while Delaware River was consistently over-represented.

Table 5 lists the estimated composition of the 1991 Virginia intercept shad fishery. Incompletely characterized shad and the nineteen percent of intercept shad whose haplotypes were unique (not traceable to in any baseline population) were withheld from the final analysis. Thus, the estimated contributions in Table 5 must be decreased by 19% to obtain the actual estimated contribution for each source population. By multiplying the maximum likelihood results in Table 5 by a factor of 0.81, it is estimated that the following populations comprised the 1991 intercept harvest: Unknown (19%), Chowan (4%), St. Johns (1%), Pamunkey (2%), Hudson (7%), Santee (16%), Connecticut (26%) and Susquehanna (25%). These estimates are depicted graphically in Figure 3.

Finally, although sample sizes precluded maximum likelihood analysis of the composition of Rudee Inlet, Chincoteague and Quinby samples individually, a qualitative analysis of their composition is in order. Examination of haplotypes other than the common AAAAAAAAAAAAAAA presented in Appendix B, indicates that most shad analyzed from Rudee Inlet shared haplotypes with Pamunkey River and a few shared haplotypes with Susquehanna River. Conversely, most of the Chincoteague and Quinby shad were typified by Susquehanna, Hudson and Santee haplotypes.

DISCUSSION

American shad examined for this study and for the larger MDNR study were of diverse genetic composition. In order to evaluate composition of the intercept fishery it was first necessary to evaluate composition of Susquehanna and other baseline stocks. Chapman and Brown (1991) reported chi-square evaluations between the baseline stocks employed here. As shown in Table 3, discrimination between populations was excellent; virtually every pairwise comparison was significantly different at $P < 0.001$. They also reported that the existing Susquehanna River stock is comprised of shad descended from native Chesapeake Bay stocks, several east coast rivers stocked into the Susquehanna system, and lineages genetically resembling the Santee River stock. In addition, while samples collected within several baseline rivers in 1990 showed no temporal genetic variation, Susquehanna samples for 1990 and 1991 were found to be significantly different from one another (chi-square = 59.92, $P<0.001$) indicating that the Susquehanna population is not in a state of genetic equilibrium. Another important finding of that sudy was that shad from upper Chesapeake Bay were genetically similar to shad of several southern lineages, particularly from Santee River.

Chi-square analysis indicated American shad landed at different Atlantic Ocean locations were significantly different as would be expected for a mixed population. Of particular interest is the fact that shad landed at Rudee Inlet were different from those landed along the Virginia portion of the Delmarva Penninsula while shad harvested along the Delmarva Penninsula by the intercept fishery (Chincoteague and Quinby) were not significantly different from one another. Since the intercept fishery is presently managed as a single unit, and since the intent of this study was to evaluate the composition of the entire intercept fishery, the three samples were not separated when conducting the maximum likelihood analysis of stock composition. Small sample sizes also precluded estimating the probable destination of shad landed at the Rudee Inlet, Chincoteague and Quinby locations separately with an acceptable degree of confidence. The final sample sizes for Rudee, Chincoteague and Quinby are small (n = 15, 40, 42, respectively) after excluding partial and unique haplotypes (17%, 7%, and 9%, respectively) and the standard deviation values for these estimates were proportionally large. However, the qualitative trend indicated by the chi-square analysis is apparent. More shad analyzed from Rudee Inlet appeared to be of Virginia origin than of Susquehanna origin, while the Chincoteague and Quinby harvests were primarily composed of lineages typifying the Susquehanna.

Nineteen percent of the intercept fish could not be classified by our current baseline data set. This indicates that either these haplotypes actually exist in the present baseline populations but were missed in the baseline samples or that intercept fish with unique haplotypes were from populations not included in the present baseline data set. The former possibility indicates that despite the highly significant chi-square values between baseline populations, these reference populations may not have been sampled intensely enough to detect all mtDNA diversity. This

possibility was examined by performing the analysis described by Hebert *et al.* (1988) for detection of clonal diversity (data not shown). For populations other than those in upper Chesapeake Bay, this analysis indicated that a sample of approximately 40 individuals was sufficient to detect a significant portion of mtDNA diversity. Therefore, as suspected, some baseline populations were inadequately sampled. The latter possibility is equally likely; other source populations which could potentially be represented in the intercept fishery were not included in the baseline data set.

Conditional maximum likelihood estimates of the composition of the remaining 81% of the intercept fishery sample indicate that those intercept shad were predominantly of Susquehanna, Connecticut, Santee and Hudson River origin (Table 5). Small contributions by other locations, from both Chesapeake Bay and from other southern rivers, were also detected in the overall analysis. Given the mixed-stock nature of the Susquehanna, the estimates reported here are actually the maximum contribution by Susquehanna River and the minimum contribution by those stocks which have been introduced to the Susquehanna system. Calculated standard deviation values were considered acceptable for the management requirements of this species and were further improved by combining estimates for stocks which were genetically similar (i.e. Susquehanna and Santee).

On first impression, the contribution by Santee River stock makes little sense. Shad from this system are not likely to be found off the Virginia coast in March while they are spawning in Santee River. Inspection of the data in Table 5 and Appendix B, however, shows that the Santee and Susquehanna Rivers share haplotypes which are not found in other populations. In addition, there is other compelling evidence to support the assumption that the estimated contribution attributed to Santee may actually be due to misclassification of Susquehanna shad. First, in a study of shad tagged off Rudee Inlet, Jesien and Hocutt (1991) report that only 17% of tag returns came from locations south of Chesapeake Bay and no returns were obtained from tributaries within the general geographic region of Santee River. Second, there is clear genetic similarity between the samples taken from Susquehanna River, Susquehanna Flats and Santee River (Chapman and Brown, 1991). Third, current knowledge of the timing of shad migration and spawning indicates that the possibility is extremely remote that the Santee stock would be found off the Virginia coast during the Spring of 1991. Other maximum likelihood analyses (data not shown) indicated that when Santee River was eliminated from the baseline data, the estimated contribution of Susquehanna River increased to 49 %. Therefore, for the present study estimated proportions of shad from Santee were combined with those for Susqehanna in order to improve estimates of contribution. Combining these groups based on patterns of similarity, suggested by Wood *et al.* (1987), changed the estimate of contribution for Susquehanna River to 41 $\pm$ 10% and significantly decreased standard deviation due to sampling variation.

CONCLUSIONS

This project involved a genetic survey to identify which, if any, target populations were harvested by Virginia's Atlantic Coast intercept fishery. The study was conducted concurrently with one of the most comprehensive investigations to date of a mixed fishery employing mtDNA analysis. The results presented here are of immediate interest to the community of managers and scientists who regulate the American shad fishery in Chesapeake Bay. Furthermore, these data constitute a minimum framework which, if expanded, will allow development of a long-term monitoring program which could eventually match that for west coast salmon in its effectiveness.

The genetic analysis provides an intitial "snapshot" of Virginia's intercept fishery indicating that approximately one-half of the shad harvested were destined for Susquehanna River. Due to the magnitude of effort and funds expended by all of the Bay states to rebuild shad stocks, policy makers and managers are sure to inquire: "Does the Virginia ocean shad harvest potentially affect the Susquehanna stock?" This question can be addressed by considering the estimate of shad harvested by Virginia's ocean fishery in 1991 (405,612 lb @ 4.5 lb per fish = 90,136 shad) multiplied by the estimated contribution of the Susquehanna to this harvest. Using the estimated 41% contribution of Susquehanna shad, it can be calculated that 36,956 ($\pm$ 3,703) shad were intercepted off the Virginia coast in 1991.** Since the total number of shad lifted over Conowingo Dam was 22,083, restriction of the intercept fishery could potentially boost the Susquehanna population by 100%. Of course, this outcome is conditioned upon the assumption that these shad would not be captured in other areas of the Bay prior to reaching the Conowingo fish lift.

One possible test of this conclusion would be to eliminate the intercept fishery for just one year and assess the increase in the Susquehanna population over and above that increase which is expected based on the present trajectory of population growth. The increase in shad lifted should be statistically analyzed to determine if the putative increase can be differentiated from the currently predicted increase without decreased fishing effort. Personnel involved with the ASMFC Shad and River Herring Scientific and Statistical Committee are familiar with shad population models and the statistics necessary to perform such an analysis.

The joint MD/VA shad tagging study at Rudee Inlet has produced some preliminary results which should be compared with the genetic data. The two studies are complementary in a very important way. Fifty-six percent of tag returns during the last six months have been from lower Chesapeake Bay; primarily from York River and its tributaries (Jesien and Hocutt, 1991). By comparison, genetic analysis estimates that more than half of the Rudee Inlet harvest is of American shad from Pamunkey River (a tributary of York River). Such close agreement indicates that the qualitative estimates of stock contribution for this location are correct.

** The 95% confidence intervals for this estimate are 19,289 and 54,622.

Another aspect of the complementary relationship between the tagging and genetic analyses is associated with the fact that no tags have been returned from upper Chesapeake Bay. There is no fishing effort for shad in upper Chesapeake Bay since both MD and PA have closed their shad fisheries. Yet the genetic analysis of the ocean catch shows an upper Bay component (Susquehanna River) in addition to a Pamunkey component. Thus, one analysis complements the other. In the future, tagging could address some critical issues raised by the genetic analysis. For example, both the Susquehanna Flats and the Susquehanna River shad populations have large Santee River components. It cannot be determined from the present data whether the Santee component of the intercept fishery is due to shad actually migrating from or to Santee River (not likely given the current state of knowledge) or whether it is due to Susquehanna shad with Santee haplotypes (very likely). If tagging endeavors are repeated once fishing resumes in upper Chesapeake Bay then both possibilities can be evaluated.

Like most other scientific investigations the present genetic analysis raises many new questions. Although the baseline data were adequate to address the majority of intercept fish harvested in 1991, they must be expanded before the entire fishery can be evaluated. We recommend increasing all baseline sample sizes to include at least 50 individuals and assessing existing Chesapeake Bay shad populations which were not examined in the present study (Rappahannock, Potomac, Patuxent, Choptank, Chester, etc.). In addition, it is clear that one sample is not adequate to formulate far-reaching management decisions. Shaklee *et al.* (1990) have examined mixed-stock fisheries of Pacific salmon. They found that stock composition varies substantially from year-to-year for mixed-stock assemblages. It would be prudent to assume that migratory mixtures of American shad stocks behave in a similar manner to Pacific coast salmonid stocks.

Depending on the Bay States' goals, we can suggest the necessary actions to undertake. First, if the sole purpose is to make an immediate management decision regarding coastal intercept shad fisheries then at least one more survey should be made of the Virginia fishery accompanied by at least two years of investigation of Maryland's ocean shad fishery. Analyses should proceed by sacrificing 150 fish per site per year, extracting mtDNA, and digesting mtDNA with the same 15 enzymes employed in the present study. The resulting haplotypes should be compared to an expanded data base. This should provide adequate information to confidently make decisions pertaining to the intercept fishery.

If goals are broader and the States wish not only to manage and regulate the intercept fisheries but to monitor all Chesapeake Bay shad populations, then we would suggest a modified research/monitoring program. That program involves conducting the research described above to address the immediate issue of the impact of the intercept fisheries. However, we would also recommend converting previously collected samples to a new format (abbreviated as "PCR") which would allow all subsequent genetic analyses to be made from material obtained by amplifying mtDNA from non-lethal biopsy tissue samples (an obvious benefit when genetic

analysis is associated with tagging). Tissue samples could be archived and analyzed at any time in the future.

Shad populations are dynamic entities influenced by both natural and anthropogenic factors. Genetic analysis offers a means not just to estimate the percent composition of mixed assemblages of shad but also to monitor the success of stocks as they respond to ecological changes and to various management practices. Like tagging, annual collection of catch-effort statistics, and other management programs, a program of genetic population analysis requires long-term commitment at the very least to collect and archive samples. Most biological monitoring programs require a minimum of four years of sequential monitoring before any type of trend can be determined. After that, analyses can be performed annually or bi-annually to re-evaluate important groups of Chesapeake Bay and intercept shad.

SIGNIFICANT POINTS

*Virginia's 1991 intercept fishery was comprised of at least 41% Susquehanna shad ($0.81 \times (31_{Sus} + 19_{Santee}\ \%)$).

*This estimate should be considered preliminary for two important reasons. First, the Susquehanna River population does not appear to have attained genetic stability and it is possible that contribution estimates for this River will vary from year to year. Second, statistical analysis indicates that several of the baseline samples are very small and may not completely represent those groups of American shad.

*Preliminary genetic analysis indicates that the Rudee Inlet harvest differs from harvests along Virginia's portion of the Delmarva Penninsula.

*These findings should be verified by a joint MD/VA investigation which would expand and enhance the current genetic baseline data set, identify specific river stocks within both states' intercept fisheries, and establish a long-term shad monitoring program (perhaps associated with joint tagging efforts).

REFERENCES

Altukhov, Y.P. and E.A. Salmenkova. 1987. Stock transfer relative to natural organization, management, and conservation of fish populations. Chapter 14 *In*: Population genetics and fishery management. N. Ryman and F. Utter, eds. Univ. of Washington Press, Seattle.

ASMFC. 1988. 1988 Supplement to American shad and river herrings fishery management plan. Fisheries Management Rept. No. 12 of the Atlantic States Marine Fisheries Commission. October 1988, Washington, DC.

ASMFC. 1990. 1990 Shad and river herring workshop, October 16-17, 1990, Nags Head, NC. Atlantic States Marine Fisheries Commission, Washington, DC.

Avise, J.C., J.E. Neigel and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. 20: 99-105.

Backman, T. National Fisheries Research and Development Laboratory, USFWS, Wellsboro, PA.

Bentzen, P., W.C. Leggett and G.G. Brown. 1988. Length and restriction site heteroplasmy in the mitochondrial DNA of American shad (*Alosa sapidissima*). Genetics 118: 509-518.

Bentzen, P., G.G. Brown and W. C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). Can. J. Aquat. Sci. 46: 1446-1454.

Birky, C.W., T. Maruyama and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103: 513-527.

Brown, B.L. and K.T. Paynter. 1991. Mitochondrial DNA analysis of native and selectively inbred Chesapeake Bay oysters, *Crassostrea virginica*. Marine Biology (in press).

CEC. 1989. Chesapeake Bay Alosid Management Plan. An agreement commitment report from the Chesapeake Executive Council. July 1989, Annapolis, MD.

Chapman, R.W. and B.L. Brown. 1990. Mitochondrial DNA isolation methods. Chapter 4 *In*: Electrophoretic and isoelectric focusing techniques in fisheries management. D.H. Whitmore, ed. CRC Press, Boca Raton, FL.

Chapman, R.W. and B.L. Brown. 1991. Genetic investigation of the resurgence of American shad, *Alosa sapidissima*, in Susquehanna River. Report submitted to Maryland Dep. of Natural Resources, Chesapeake Bay Research and Monitoring Division, Annapolis, MD.

Chapman, R.W. and D.A. Powers. 1984. A method for rapid isolation of mitochondrial DNA from fishes. Maryland Sea Grant Tech. Rept. No. UM-SG-TS-84-05.

Crow, J.F. and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row. NY.

Dadswell, M.J., G.D. Melvin and P.J. Williams. 1983. Effect of turbidity on the temporal and spatial utilization of the inner Bay of Fundy by American shad (*Alosa sapidissima*) (Pisces:Clupeidae) and its relationship to local fisheries. Can. J. Fish. and Aquat. Sci. 40 (Suppl. 1): 322-330.

Fisher, R.A. 1958. The genetical theory of natural selection. Dover Press, NY.

Flagg, L. 1990. Review of Atlantic States Marine Fisheries Commission Fishery Management Plan for American shad and river herring. pp 76-82 *In*: Atlantic States Marine Fisheries Commission 1990 shad and river herring workshop. Oct. 16-17, 1990, Nags Head, NC.

Fournier, D.A., T.D. Beacham, B.E. Riddell and C.A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. and Aquat. Sci. 41: 400-408.

Gibson, M., V. Crecco an dD. Stang. 1988. Stock assessment of American shad from selected Atlantic coast rivers. Special Report No. 15 of the Atlantic States Marine Fisheries Commission, Washington, DC.

Gibson, M. Rhode Island Div. of Fish and Wildlife, W. Kingston, RI.

Hebert, P.D., R.D. Ward and L.J. Weider. 1988. Clonal-diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual-sexual complex. Evolution 42: 147-159.

Jesien, R. and C. Hocutt. 1991. Movement of American shad in the Chesapeake Bay. Progress Report to MDNR, Annapolis, MD.

Leggett, W.C. and R.R. Whitney. 1972. Water temperature and the migration of American shad. Fish. Bull. (US) 70:659-670.

Melvin, G.D., J.D. Michael and J.D. Martin. 1985. Fidelity of American shad, *Alosa sapidissima*, (Osteichthyes:Clupeidae) to its river of previous spawning. Can. J. Fish. and Aquat Sci. 42:1-19.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Pella, J.J. 1986. The method of fitting expectations applied to computation of conditional maximum likelihood estimates of stock composition from genetic marks. US DOC/NOAA/NMFS/NWAFC Auke Bay Laboratory, Auke Bay, AK. Rept. No. 171.

Pella, J.J. and G.B. Milner. 1987. Use of genetic marks in stock composition analysis. Chapter 10 *In*: Population genetics and fishery management. N. Ryman and F. Utter, eds. Univ. of Washington Press, Seattle.

Roff, D.A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: Chi-square and the problem of small samples. Mol. Biol. Evol. 6: 539-545.

Shaklee, J. C. Busack, A. Marshall, M. Miller and S. Phelps. 1990. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon. pp. 235-265 *In*: (Z-I. Ogita and C. Marke, eds.) Isozymes: Structure, Function, and Use in Biology and Medicine. Prog. in Clin. and Biol. Res. Vol. 344. Wiley-Liss, Inc. New York.

Sokal, R.R. and F. J. Rohlf. 1981. Biometry. W.H. Freeman and Co., San Francisco.

Stagg, C. 1986. An evaluation of the information available for managing Chesapeake Bay fisheries: preliminary stock assessments, Vol. I and II. Univ. Maryland, CBL, UMCEES [CBL] Ref. No. 85-29.1.

Takahata, N. and T. Maruyama. 1981. A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res., Camb. 37: 291-302.

Walburg, C.H. and R.P. Nichols. 1967. Biology and management of the American shad and status of the fisheries. Atlantic coast of the United States 1960. US Fish and Wildl. Ser. Special Sci. Rept. Fish. No. 550.

Wood, C.C., S. McKinnell, T.J. Mulligan and D.A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can J. Fish. Aquat. Sci., 44: 866-881.

Figure 1. Map of the Chesapeake Bay region showing locations of American shad samples taken by commercial fishermen in 1991.

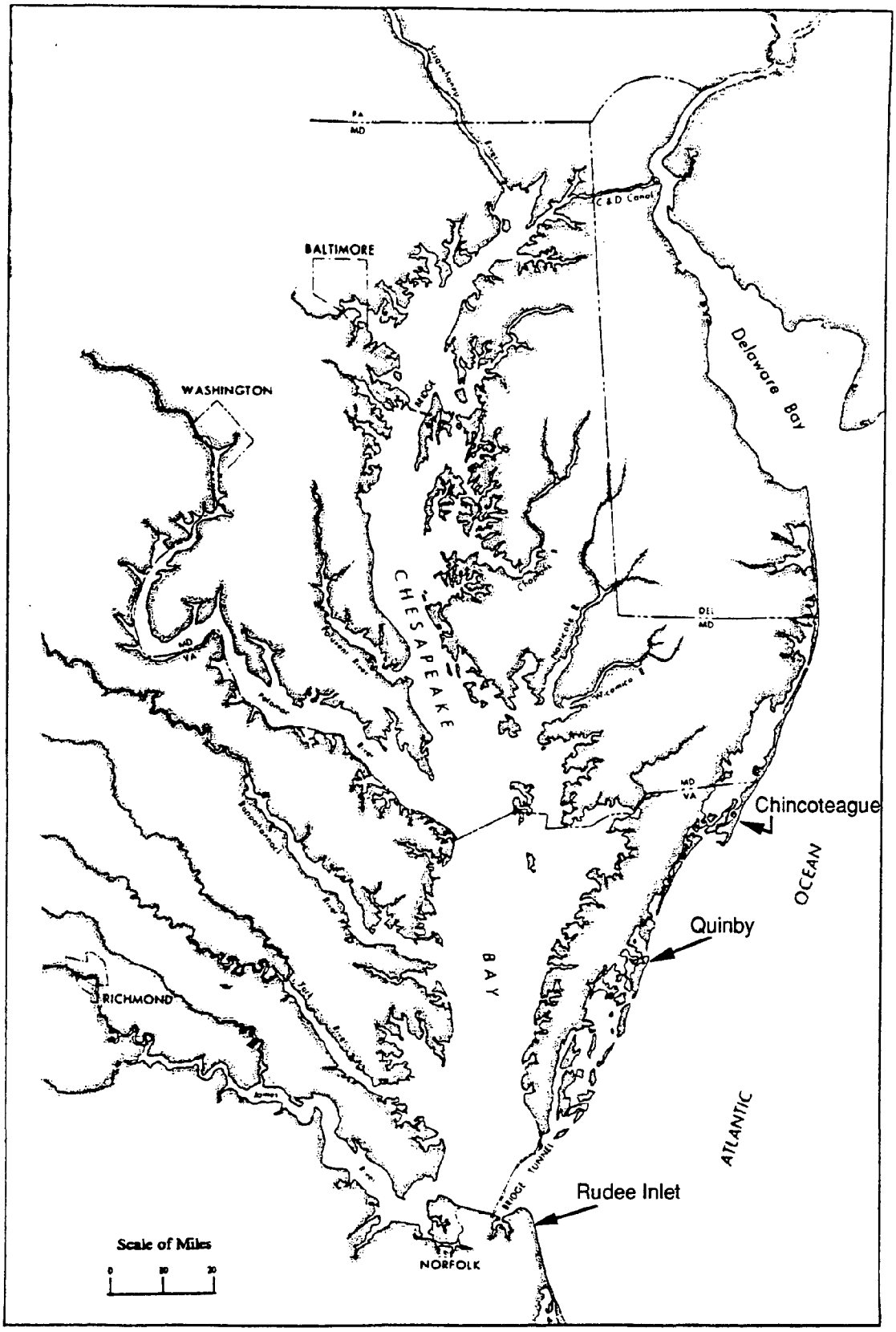
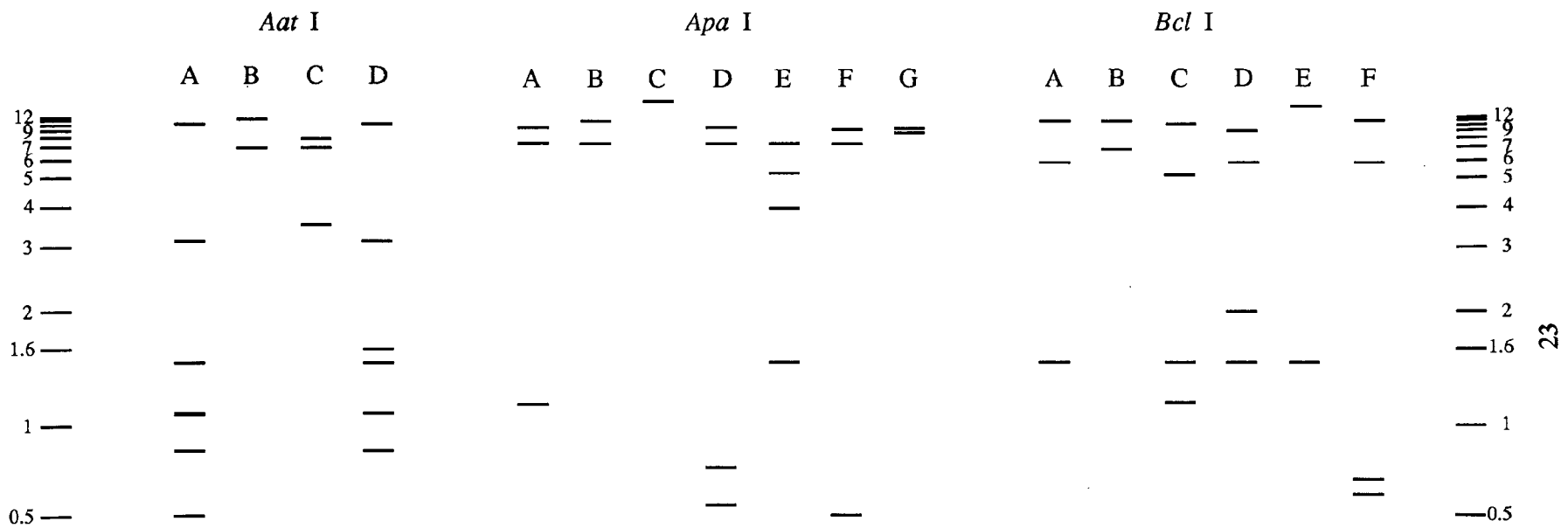
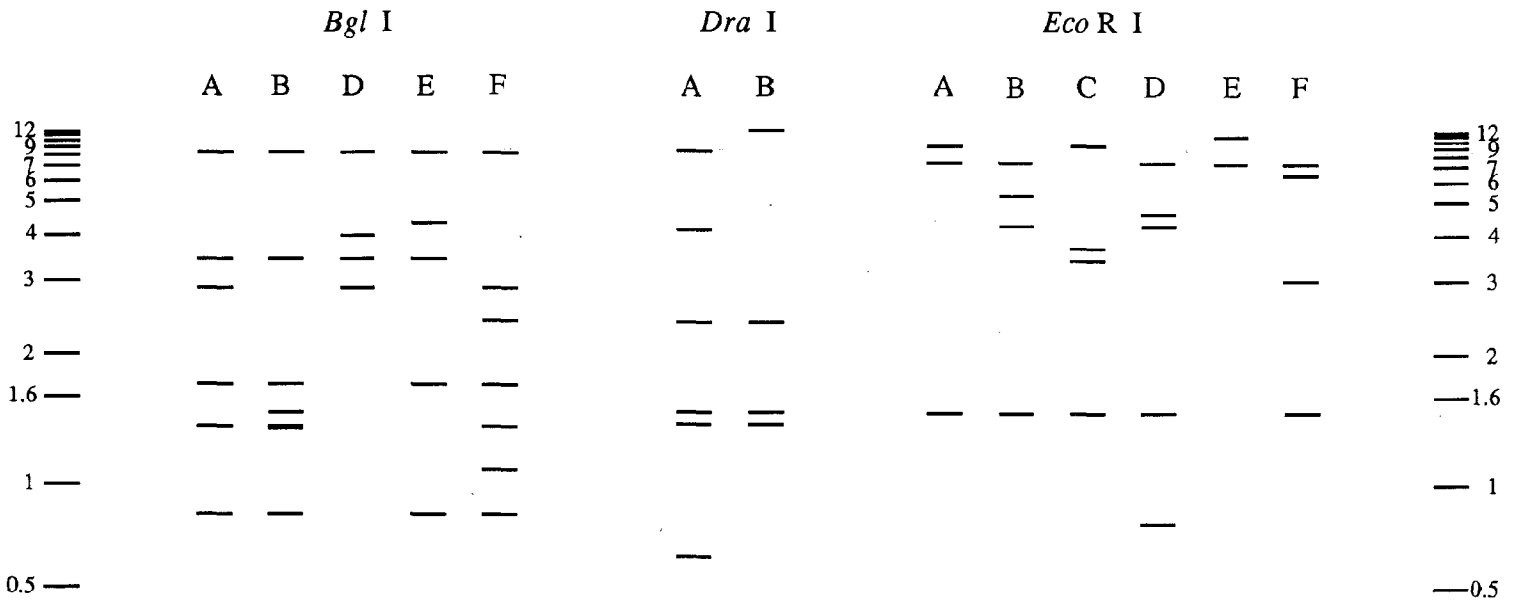
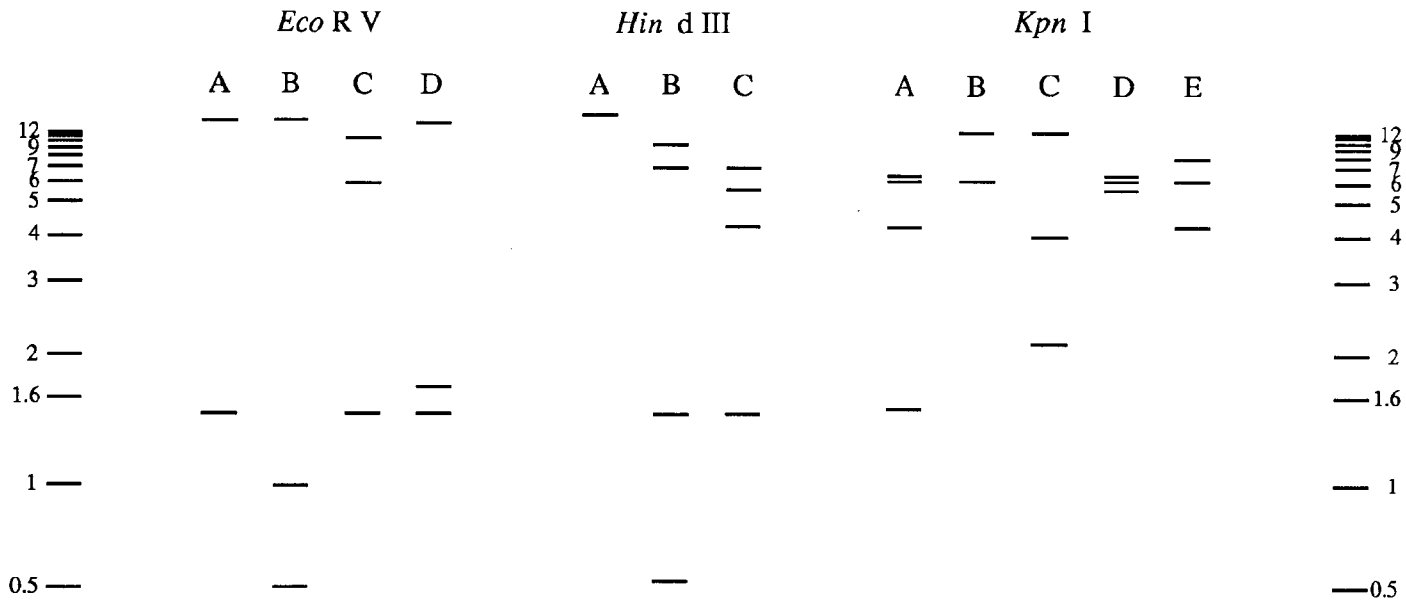
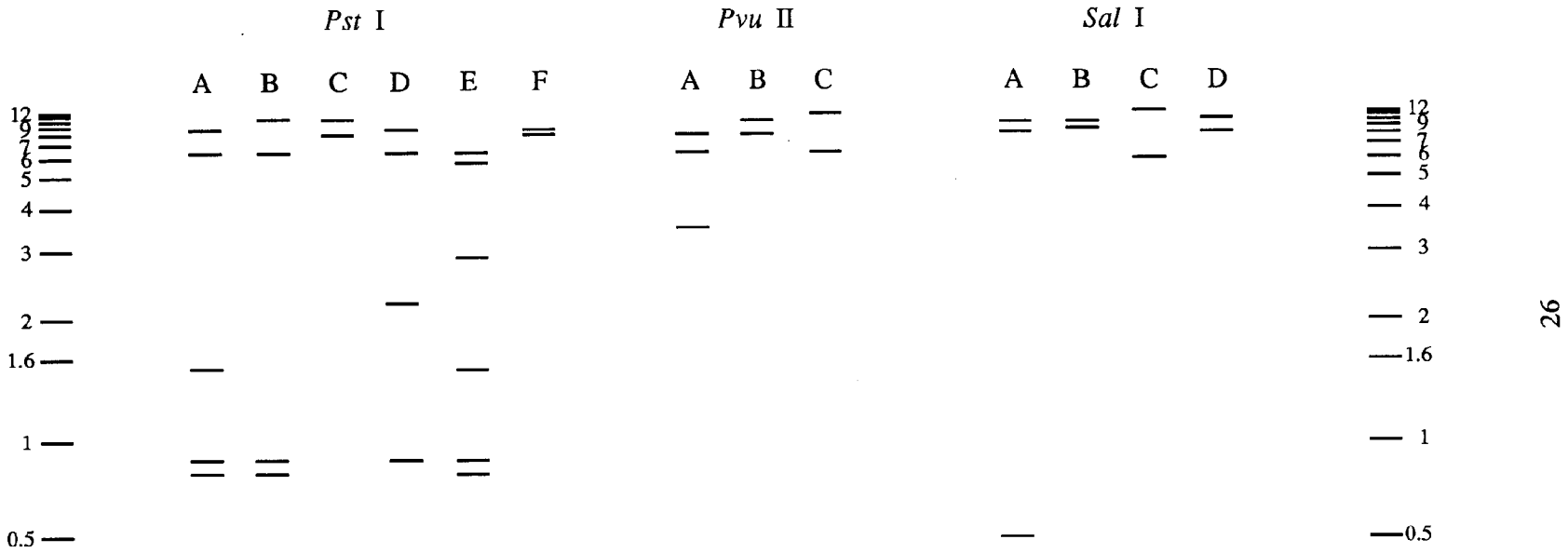

BALTIMORE
WASHINGTON
RICHMOND
NORFOLK
CHESAPEAKE
BAY
C&D Canal
Delaware Bay
OCEAN
ATLANTIC
Chincoteague
Quinby
Rudee Inlet
Scale of Miles

Figure 2. Graphic depiction of restriction fragment patterns for American shad.


Aat I
A
B
C
D
12
9
7
6
5
4
3
2
1.6
1
0.5
Apa I
A
B
C
D
E
F
G
Bcl I
A
B
C
D
E
F
12
9
7
6
5
4
3
2
1.6
1
0.5


Bgl I
A B D E F
Dra I
A B
Eco R I
A B C D E F
12
9
7
6
5
4
3
2
1.6
1
0.5


Eco R V
Hin d III
Kpn I
A
B
C
D
A
B
C
A
B
C
D
E
12
9
7
6
5
4
3
2
1.6
1
0.5


Pst I
A
B
C
D
E
F
Pvu II
A
B
C
Sal I
A
B
C
D
12
9
7
6
5
4
3
2
1.6
1
0.5

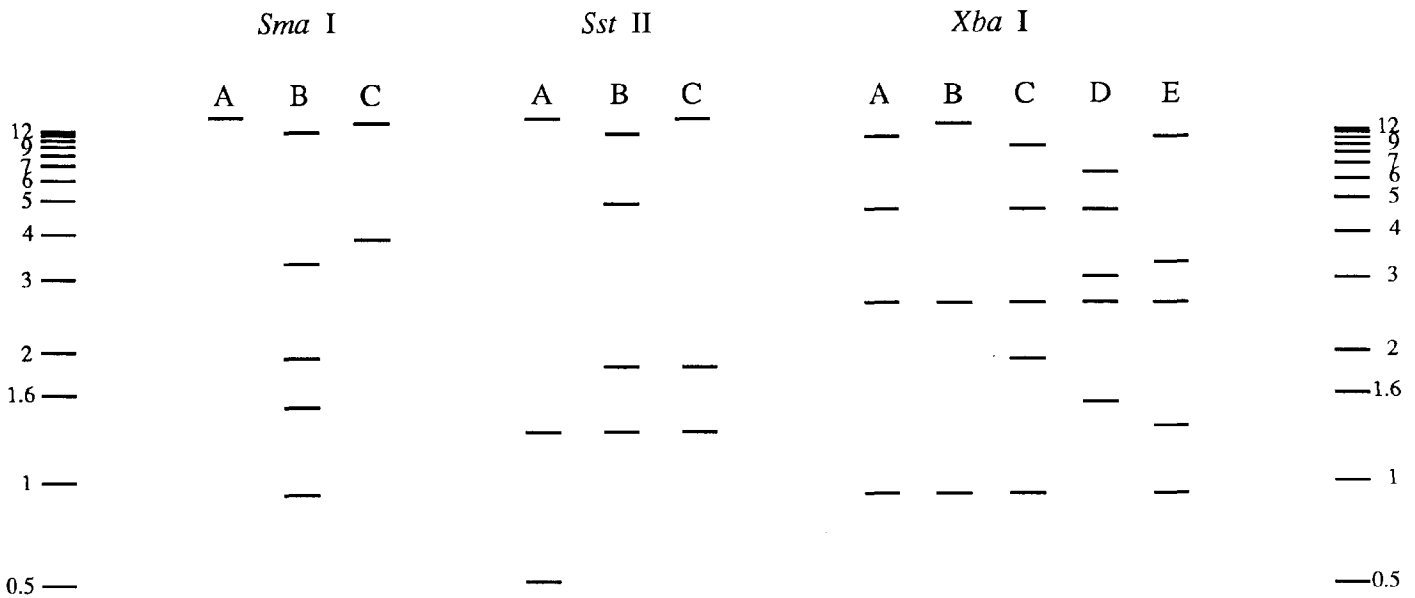

Sma I
Sst II
Xba I
A
B
C
A
B
C
A
B
C
D
E
12
9
7
6
5
4
3
2
1.6
1
0.5
12
9
7
6
5
4
3
2
1.6
1
0.5

Figure 3. Diagram of estimated contribution by baseline populations to Virginia's 1991 Atlantic Ocean harvest of American shad.

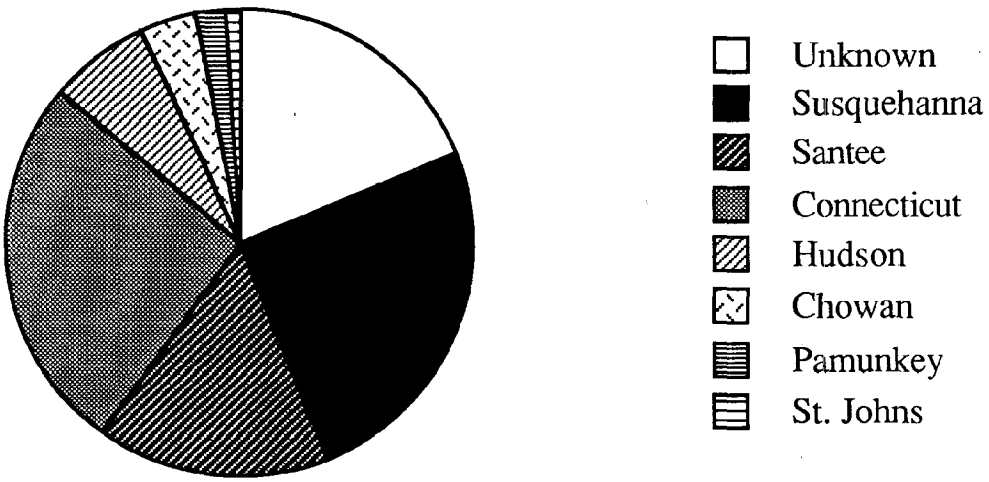


Table 1. Summary, by river of origin, of American shad stocked into the Susquehanna River during the period 1982–1991. Shad from Columbia River, WA are an introduced population derived from the Hudson River stock. Information provided by the Susquehanna River Coordinator.

A. Hatchery cultured fry (millions)

Year	Pamunkey	James	Columbia	Delaware	Hudson
1991	–	–	–	3.212	8.845
1990	0.178	–	–	3.565	6.000
1989	0.754	0.220	12.422	1.645	5.660
1988	0.655	0.029	8.467	0.949	–
1987	1.403	0.040	6.919	1.227	–
1986	2.433	0.210	11.184	1.243	–
1985	2.222	0.458	1.906	1.642	–
1984	4.289	0.509	7.162	0.380	–
1983	1.000	1.100	1.950	–	–
1982	1.151	0.740	6.949	–	–

B. Live pre–spawned adults

Year	Susquehanna	Hudson	Connecticut
1991	22083	–	–
1990	14792	–	–
1989	6590	–	–
1988	4730	–	–
1987	6900	6032	–
1986	4080	4965	–
1985	950	3158	64
1984	0	3592	185
1983	0	3123	1187
1982	875	992	1573

Table 2. Mitochondrial DNA haplotypes of American shad harvested outside Chesapeake Bay in 1991. Haplotypes are created by listing single–enzyme genotypes. The order of enzymes in a haplotype is as listed on p. 6. Genotype assignments in the form "A/B" or "A/C" are heteroplasmic combinations of genotypes "A and B" or "A and C", respectively.

Haplotype	Site		
	Rudee	Chincoteague	Quinby
AAAAAAAAAAAAAAA	2	16	14
AAAAAAAAAAAAAAA/B	0	1	0
AAAAAAAAAAAAAAB	0	1	0
AAAAAAAAAAABAAA	0	1	1
AAAAAAAAAABAAAA	0	1	1
AAAAAAAAA/BAAA/BAAA	0	0	1
AAAAAAAABAAAAAA	1	4	5
AAAAAAAABAAAAAB	1	0	0
AAAAAAAABAAAABA	1	0	0
AAAAAAAABAABAAA	1	7	3
AAAAAAAABEAAAAA	1	0	0
AAAAAAAACAAAAAA	0	0	1
AAAAAABABAAAAAA	0	0	1
AAAAAABABAABAAA	0	0	1
AAAAAACAAAAA/CAAB	1	0	0
AAAAAACAAAACAAA	1	0	0
AAAAAACABAAAAAA	1	0	0
AAAAAACABAAAAAB	1	0	0
AAAAAACABAAA/CAAB	1	0	0
AAAAABAAAAAAAAA	0	1	0
AAAABAAAAAAAAAA	0	1	0
AAAFAAAAAAAAAAA	1	0	0
ABAAAAAAAAAAAAA	0	1	0
AGAAAAAAAAABAAA	0	1	0
BABAAAAAEAAAAAA	0	0	1
DAAAAAAAAAAAAAA	0	5	8
DAAAAAAAAABAAAA	0	0	2
DAAAAAAABAAAAAA	0	0	1
DAAAAAAABAABAAA	0	0	1
DAAAAAA/CAAA/CAAAAA	1	0	0
DAAAAABAAAAAAAA	0	0	1
DAAAAABAAAAA/CAAB	1	0	0
Totals	15	40	42

Table 3. Chi–square comparisons for American shad in baseline populations calculated per Roff and Bentzen (1989). The first entry in each cell is the observed chi–square value, the second entry is the largest chi–square obtained by simulation, and the third entry is the probability that the observed value was due to chance. Rivers are abbreviated as follows: S90–Susquehanna at conowingo Dam in 1990, S91–Susquehanna at Conowingo Dam in 1991, JAM–James, NAN–Nanticoke, SF–Susquehanna Flats, PAM–Pamunkey, CHO–Chowan, DEL–Delaware, CT–Connecticut, SAV–Savannah, STJ–St. Johns, HUD–Hudson, SAN–Santee, COL–Columbia. Data are from Chapman and Brown (1991).

	S91	JAM	NAN	SF	PAM	CHO	DEL	CT	SAV	STJ	HUD	SAN	COL
S90	59.92 39.17 0.000	87.29 32.17 0.000	58.34 29.20 0.000	34.29 38.83 0.001	69.98 35.20 0.000	31.90 34.54 0.001	60.33 23.38 0.000	40.76 30.79 0.000	103.02 31.32 0.000	70.33 39.48 0.000	69.98 27.67 0.000	62.64 31.08 0.000	116.44 27.76 0.000
S91	--	39.06 29.40 0.000	35.55 30.46 0.000	11.07 25.43 0.362	30.70 23.18 0.000	16.08 23.38 0.052	31.89 29.76 0.000	18.52 21.31 0.003	51.62 29.40 0.000	34.33 25.75 0.000	34.43 33.82 0.000	30.60 26.26 0.000	67.05 28.58 0.000
JAM	--	--	49.88 30.81 0.000	27.59 25.93 0.000	49.13 26.14 0.000	29.72 25.72 0.000	57.50 28.56 0.000	33.55 20.20 0.000	56.85 31.65 0.000	40.92 24.43 0.000	54.69 31.07 0.000	50.24 30.16 0.000	83.01 35.61 0.000
NAN	--	--	--	19.53 21.85 0.004	37.36 26.68 0.000	23.62 21.88 0.000	52.13 30.90 0.000	38.00 20.78 0.000	60.18 39.00 0.000	38.98 29.06 0.000	47.92 29.78 0.000	48.21 29.59 0.000	69.06 29.68 0.000
SF	--	--	--	--	16.51 22.70 0.013	8.57 11.24 0.038	17.38 27.64 0.089	10.47 17.32 0.024	39.11 32.74 0.000	22.64 20.39 0.000	18.78 25.19 0.000	15.94 23.50 0.047	56.12 23.40 0.000
PAM	--	--	--	--	--	22.05 19.75 0.000	51.72 26.79 0.000	32.20 21.83 0.000	62.72 33.09 0.000	43.32 22.31 0.000	45.98 31.23 0.000	45.13 24.64 0.000	76.20 30.21 0.000
CHO	--	--	--	--	--	--	16.36 34.73 0.117	10.69 15.01 0.020	43.46 28.42 0.000	21.34 19.34 0.000	22.30 31.36 0.009	15.58 22.68 0.028	56.98 27.59 0.000
DEL	--	--	--	--	--	--	--	12.52 25.02 0.297	68.90 30.10 0.000	42.12 27.41 0.000	41.17 35.39 0.000	31.94 28.86 0.000	94.22 32.98 0.000
CT	--	--	--	--	--	--	--	--	48.83 24.77 0.000	26.82 17.84 0.000	22.05 21.35 0.000	18.60 20.85 0.004	69.00 22.72 0.000
SAV	--	--	--	--	--	--	--	--	--	51.61 28.11 0.000	69.79 36.63 0.000	63.48 30.34 0.000	90.50 28.43 0.000
STJ	--	--	--	--	--	--	--	--	--	--	45.98 31.28 0.000	42.44 24.71 0.000	71.00 24.02 0.000
HUD	--	--	--	--	--	--	--	--	--	--	--	36.22 27.82 0.000	79.56 26.06 0.000
SAN	--	--	--	--	--	--	--	--	--	--	--	--	83.00 28.06 0.000

Table 4. Results of a typical maximum likelihood analysis of simulated mixture populations. Tests were conducted to determine ability of the GIRLSEM algorithm to estimate stock composition of a mixed sample from shad mtDNA data. Mixture populations of known composition were created by randomly sampling twelve baseline samples with replacement. Final sample size for the analysis shown was 96. Data are taken from Chapman and Brown (1991) which included Columbia River, WA (denoted COL) as one of the baseline samples.

Source	Contribution			Ho:A=E	alpha=
	Actual	Estim.	SE	z	0.05
1. COL	0.066	0.067	0.036	0.016	accept
2. CT	0.066	0.000	0.025	−2.604	reject
3. DEL	0.104	0.359	0.058	4.405	reject
4. HUD	0.142	0.149	0.051	0.156	accept
5. SF	0.028	0.000	0.017	−1.672	accept
6. NAN	0.066	0.074	0.037	0.222	accept
7. PAM	0.076	0.066	0.037	−0.243	accept
8. JAM	0.113	0.059	0.040	−1.353	accept
9. CHO	0.047	0.000	0.022	−2.181	reject
10. SAN	0.113	0.082	0.043	−0.718	accept
11. SAV	0.094	0.074	0.040	−0.500	accept
12. STJ	0.085	0.068	0.038	−0.428	accept

Table 5. Estimated stock composition of three groups of American shad. A) Virginia's intercept fishery and B) Susquehanna River in 1990 and in 1991. Abbreviations are as listed in Table 3.

A. Composition estimates for 81% of Virginia's 1991 intercept fishery. Nineteen percent of intercept shad could not be characterized by the current baseline data. Columbia River was not included in the maximum likelihood analysis of Virginia's fishery because that stock would not be represented. Fish of Columbia River descent are allocated by the analysis to contributions by Susquehanna and Hudson Rivers.

Source	Contribution	SD
CT	0.32	0.11
DEL	0.00	0.00
HUD	0.09	0.05
SF	0.00	0.00
NAN	0.00	0.00
PAM	0.02	0.02
JAM	0.00	0.00
CHO	0.05	0.05
SAN	0.19	0.12
SAV	0.00	0.00
STJ	0.02	0.02
SUS	0.31	0.10

B. Estimated composition of Susquehanna River shad samples collected in 1990 and 1991. Forty-eight percent of shad taken in 1990 and 31% of shad in 1991 were unique to the Susquehanna system. Composition estimates are given for the remaining 52% and 69%, respectively. The Columbia River shad stock (denoted COL) was included in the analysis of Susquehanna shad because that stock has been introduced into the Susquehanna system.

	1990		1991	
Source	Contribution	SD	Contribution	SD
COL	0.01	0.01	0.04	0.04
CT	0.03	0.06	0.50	0.13
DEL	0.00	0.00	0.00	0.00
HUD	0.00	0.00	0.00	0.00
SF	0.03	0.08	0.02	0.10
NAN	0.16	0.06	0.00	0.00
PAM	0.03	0.03	0.06	0.06
JAM	0.00	0.00	0.06	0.06
CHO	0.12	0.10	0.12	0.10
SAN	0.14	0.07	0.00	0.00
SAV	0.00	0.00	0.00	0.00
STJ	0.00	0.00	0.00	0.00

Appendix A. Genotypes of American shad taken in the coastal intercept fishery outside Chesapeake Bay during the Spring of 1991. Enzyme titles are abbreviated versions of those listed on p. 6. Genotypes given with a "/" represent heteroplasmic combinations of both genotypes listed. A dash "–" indicates incomplete data for determining that fish's genotype.

		Enzyme Genotype														
Location	ID	Aat	Apa	Bcl	Bgl	Dra	EcI	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Rudee Inlet	1	D	A	A	A	A	A	B	A	A	A	A	A/C	A	A	B
	2	D	A	A	A	A	A	A/C	A	A	A/C	A	A	A	A	A
	3	–	B	A	A	A	A	A	A	A	B	A	A	–	–	A
	4	–	A	A	–	A	A	A	A	–	A	A	–	–	–	–
	5	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A
	6	–	B	–	A	–	A	–	–	A	B	–	B	A	A	B
	7	–	A	–	–	–	A	–	A	A	–	A	–	–	–	A
	8	–	–	–	–	A	A	A	–	A	A	A	–	–	–	A
	9	–	A	–	A	–	A	–	–	A	–	–	–	–	–	A
	10	–	–	A	–	–	A	–	–	A	–	A	A	–	–	–
	11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	12	–	A	A	A	–	A	–	A	A	A	A	A	–	–	–
	13	–	–	–	–	–	A	–	A	A	A	A	A	–	–	–
	14	A	–	A	–	A	A	–	A	A	A	A	A	–	–	A
	15	A	–	A	A	A	A	A	A	–	A	A	–	–	A	A
	16	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	17	–	A	A	A	–	A	–	A	A	A	A	A	–	A	A
	18	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	19	–	A	A	–	–	A	–	–	A	A	A	A	–	A	A
	20	–	–	–	–	–	A	–	–	A	A	A	–	–	–	–
	21	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–
	22	–	A	A	A	–	A	–	A	A	A	A	A	–	A	A
	23	–	–	–	A	–	A	–	A	A	–	–	–	–	–	A
	24	–	A	–	A	–	B	–	A	A	–	–	–	–	–	A
	25	A	A	A	A	A	A	C	A	A	A	A	C	A	A	A
	26	A	A	–	A	A	–	–	–	A	–	–	–	–	–	A
	27	A	–	–	A	A	A	–	–	A	A	A	–	–	–	–
	28	A	–	–	A	A	A	–	A	A	–	–	–	–	–	A
	29	A	A	A	A	A	A	C	A	A	A	A	A/C	A	A	B
	30	A	–	–	A	A	A	–	–	A	–	–	–	–	–	A
	31	D	–	A	A	A	A	–	–	A	A	–	A	–	–	A
	32	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A
	33	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–
	34	A	A	A	A	A	A	C	A	B	A	A	A	A	A	A
	35	A	–	–	A	A	A	–	–	A	–	–	–	–	–	A
	36	A	A	A	A	A	–	C	A	B	A	A	A/C	–	A	B
	37	A	A	A	A	A	A	–	–	A	A	A	A	–	–	A
	38	A	–	–	A	A	–	–	A	A	A	A	–	–	–	A
	39	–	A	–	A	A	A	–	A	B	A	A	A	–	A	–
	40	A	A	A	A	A	A	C	A	B	A	A	A	A	A	B
	41	A	A	A	A	A	A	A	A	B	E	A	A	A	A	A
	42	A	A	A	A	A	–	A	A	B	A	A	A	–	A	B
	43	–	A	A	A	A	–	A	A	B	A	A	B	–	A	–
	44	–	A	A	A	A	A/B	A	A	B	A	A	A	–	A	–
	45	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B
	46	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–
	47	–	A	A	A	–	–	A	A	B	–	A	A	–	–	–
	48	–	–	–	A	–	–	–	A	–	–	–	–	–	–	–
	49	A	–	A	A	A	–	A	–	A	A	–	–	–	A	–
	50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	52	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Appendix A. Continued

Location	ID	Enzyme Genotype														
		Aat	Apa	Bcl	Bgl	Dra	EcI	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Chincoteague	1	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
	2	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	4	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	5	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A
	6	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	7	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	8	–	–	A	A	A	A	A	A	–	A	A	A	A	A	–
	9	–	–	A	A	A	A	A	A	–	A	A	A	A	A	–
	10	–	A	A	A	A	A	A	A	–	A	–	B	–	A	–
	11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/B
	13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	14	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
	16	–	–	A	A	A	A	A	A	–	A	A	A	–	A	–
	17	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	18	–	A	A	A	A	A	A	A	A	A	–	–	–	A	–
	19	–	A	A	A	A	A	A	A	A	A	–	–	A	A	–
	20	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	21	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	23	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	24	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	26	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	27	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	28	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
	29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	30	A	A	A	A	A	–	–	–	A	–	A	A	A	A	–
	31	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–
	32	–	A	A	–	–	–	–	–	–	–	A	A	–	A	–
	33	–	A	A	–	–	–	–	–	–	–	A	B	–	A	–
	34	A	G	A	A	A	A	A	A	A	A	A	B	A	A	A
	35	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A
	36	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	37	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	38	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	39	–	–	–	–	–	–	–	–	A	–	A	A	–	–	–
	40	–	–	A	–	–	–	–	–	–	–	A	A	–	A	–
	41	A	A	A	A	A	–	–	–	B	A	A	B	A	A	A
	42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	43	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	44	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	45	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	47	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	48	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	49	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	50	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	51	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	52	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	53	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	54	–	A	A	A	A	–	A	A	–	A	–	A	A	A	–

Appendix A. Continued

Location	ID	Enzyme Genotype														
		Aat	Apa	Bcl	Bgl	Dra	EcI	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Quinby	1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	2	–	A	–	–	–	–	–	–	A	–	A	A	–	–	–
	3	–	A	A	A	–	–	–	A	A	A	A	A	A	A	A
	4	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	6	–	–	–	–	–	–	–	A	B	A	A	A	A	A	–
	7	A	A	A	A	A	A	B	A	B	A	A	A	A	A	A
	8	A	A	–	–	–	–	–	A	A	A	–	A	A	A	A
	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	A	A	A	A	A	A	A	A	A/B	A	A	A/B	A	A	A
	11	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	12	A	A	A	A	A	A	B	A	B	A	A	B	A	A	A
	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–
	15	–	A	–	–	–	A	–	–	–	–	–	A	–	–	A
	16	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–
	17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	18	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	19	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	20	–	A	–	–	–	–	–	A	A	A	A	A	A	A	A
	21	–	A	A	A	A	–	–	–	A	A	A	A	–	–	A
	22	A	A	A	A	A	–	A	A	C	A	A	A	A	A	A
	23	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	24	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	25	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	26	D	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	27	D	A	A	A	A	A	B	A	A	A	A	A	A	A	A
	28	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	30	D	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	31	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	32	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	33	A	A	A	A	A	–	A	A	A	A	A	A	–	A	–
	34	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	35	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	36	–	–	–	A	A	–	–	A	–	A	A	A	A	A	A
	37	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	38	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	39	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	40	A	–	A	A	–	A	A	–	–	A	A	A	–	A	–
	41	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	43	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	44	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	45	B	A	B	A	A	A	A	A	E	A	A	A	A	A	A
	46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	47	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A
	48	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	49	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	50	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	51	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	52	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	53	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	54	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	55	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	56	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Appendix B. Distribution of mtDNA haplotypes in intercept and baseline American shad population samples. Abbreviations for the intercept samples are Rudee Inlet–R, Chincoteague–C, and Quinby–Q. Other abbreviations are as listed in Table 3.

		Population																
	Haplotypes	R	C	Q	S90	S91	SF	COL	CT	DEL	HUD	NAN	PAM	JAM	CHO	SAN	SAV	STJ
1	AAAAAAAAAAAAAAA	2	16	14	17		6		15	22	14	3	5	5	9	14	3	6
2	AAAAAAAAAAAAAAB		1			1												
3	AAAAAAAAAAAAAAA/B		1															
4	AAAAAAAAAAAAAAD															3		
5	AAAAAAAAAAAAABA				3					1		1						
6	AAAAAAAAAAAAABA/B				1													
7	AAAAAAAAAAAAABD											1						
8	AAAAAAAAAAAABAA											1		1			1	3
9	AAAAAAAAAAAABBA													3			9	
10	AAAAAAAAAAAACAA																1	
11	AAAAAAAAAAABAAA		1	1	2					2		1			1	4		
12	AAAAAAAAAAABABA					1												
13	AAAAAAAAAAACBBA																1	
14	AAAAAAAAAAADAAA																1	
15	AAAAAAAAAABAAAA		1	1						1	4							
16	AAAAAAAAAABAABA													1				
17	AAAAAAAAAABABBA																1	
18	AAAAAAAAAA/BAAAAA				1													
19	AAAAAAAAAA/CAAAAA				1													
20	AAAAAAAAAA/CABAAA/B				1													
21	AAAAAAAAACAAAAA									1								
22	AAAAAAAAACAAAAA													8				1
23	AAAAAAAAADAAAAA								1									
24	AAAAAAAAAEAAAAA																	1
25	AAAAAAAAAFAAAAA															1		
26	AAAAAAAAA/BAAA/BAAA			1														
27	AAAAAAAABAAAAAA	1	4	5	5				6	8	2		1	3		2	2	2
28	AAAAAAAABAAAAAB	1											2	1	2			
29	AAAAAAAABAAAABA	1				3												
30	AAAAAAAABAAABBA									1							6	
31	AAAAAAAABAAACBA																1	
32	AAAAAAAABAABAAA	1	7	3		2	2		2	2	5		2	2		7	1	
33	AAAAAAAABAABABA					1						1						
34	AAAAAAAABAABACA															1		
35	AAAAAAAABAABBAA																	1
36	AAAAAAAABAABBBA									1							1	
37	AAAAAAAABCAAAAA													1				
38	AAAAAAAABCAABBA													1				
39	AAAAAAAABCBBAAA													1				
40	AAAAAAAABC/AABAAA													1				
41	AAAAAAAABFAABBA																1	
42	AAAAAAAACAABAAA													1				
43	AAAAAAAACAABBBA																1	
44	AAAAAAAADAAAAAA									1								
45	AAAAAAAADAAAABA											1						
46	AAAAAAAAECAAAAA				1													
47	AAAAAAABAAAAABA										1							
48	AAAAAAABAAAABBA																1	
49	AAAAAAABEAAAAAA	1			1													
50	AAAAAAACAAAAAAA			1							1							
51	AAAAAAA/CAAAAABAA/B				1													
52	AAAAAAA/CABAAAAAA				1													
53	AAAAAAA/CABEAAAAA				1													

Appendix B. Continued

	Haplotypes	Population																
		R	C	Q	S90	S91	SF	COL	CT	DEL	HUD	NAN	PAM	JAM	CHO	SAN	SAV	STJ
54	AAAAAABAAAAAAAA				3						4	3	1					
55	AAAAAABAAAABAAB											1						
56	AAAAAABAAACAAAA										2							
57	AAAAAABAAA/CAAABA				1													
58	AAAAAABABAAAAAA			1	1													
59	AAAAAABABAABAAA			1														
60	AAAAAACAAAAAAAA				4		2			1		4	3					
61	AAAAAACAAAAAAAA/B				1													
62	AAAAAACAAAAAAAD				1													
63	AAAAAACAAAAABBA											1						
64	AAAAAACAAAABAAA												1					
65	AAAAAACAAAACAAA	1																
66	AAAAAACAAAAA/CAAB	1																
67	AAAAAACABAAAAAA	1											2					
68	AAAAAACABAAAAAB	1																
69	AAAAAACABAAAABA												1					
70	AAAAAACABAABAAA												2					
71	AAAAAACABAABAAA												1					
72	AAAAAACACAABAAA												1					
73	AAAAAACABAAA/CAAB	1																
74	AAAAAACBAAAAAAA													1				
75	AAAAAACBAAAAABD				1													
76	AAAAAACBAAAABBA											1		1				
77	AAAAAACBAAACBBA											1						
78	AAAAAACBACAAABA													1				
79	AAAAAACBBAAAABA																1	
80	AAAAAACBBAAC/BBBA											1						
81	AAAAAACBDAAAAAA											1						
82	AAAAAACCAAAAAAA													1				
83	AAAAAACCAAAAABA				1													
84	AAAAAACCAAAABBA											1						
85	AAAAAACCCAACBBA											1						
86	AAAAAADBBAAAABA				1													
87	AAAAABAAAAAAAAA		1															2
88	AAAAABAAAA/CAAAAA				1													
89	AAAAABAABAAAAAA															1		
90	AAAABAAAAAAAAAA		1															
91	AAAABBAAAAAAAAA													1				
92	AAAA/EAAAAAAAAAAA							26			2							
93	AAAA/EAAAABAAAAAA										3							
94	AAAA/EAABAAAAAAAA							3										
95	AAAA/EAACAAAAAAAA							5										
96	AAAAA/EACAAAAAAAA				1													
97	AAABAAAAAAAAAAA												1					
98	AAABAAAAAAAABAA									1								
99	AAABAACAAAAAAAA												1					
100	AAACAAAAAAABBBA																1	
101	AAACAAAABAAABBA																1	
102	AAAEAAAAAAAAAAA							1										
103	AAAEAACCABAAAAA							1										
104	AAAFAAAAAAAAAAA	1																
105	AABAAAAABAAAAAA									1						1		
106	AACAAAAAAAAAAAA									1								
107	AACAAAAAAABAAAA										1							
108	AADAAAAAAAAAAAA															2		
109	AADAAAAABAAAABA				1													
110	AADAAAAAAAAABBA													1				
111	AADAAAAACCABAAC													1				

Appendix B. Continued

	Haplotypes	Population																
		R	C	Q	S90	S91	SF	COL	CT	DEL	HUD	NAN	PAM	JAM	CHO	SAN	SAV	STJ
112	AAEAAAAAAAAAAAA															1	1	
113	AAEAAAAAACABBAA																1	
114	ABAAAAAAAAAAAAA		1															
115	ABAAAAAAAAAABAA									2								
116	ABAAAAAAAAAABAA																	3
117	ABAAAAAAAAACAAA									1								
118	ABAAAAAAACAAAAA													2				
119	ABAAAAAABBAAABA					1												
120	ABAAAACAAABAAAA												1					
121	ABAAAACABBAAAAA												1					
122	ABAAABAAAAAABAA																	1
123	ABACAAAAACAAAAA									1								
124	ACAAAAAAAAAAAAA																1	3
125	ACAAAAAAAAAABAA																	1
126	ACAAAAAAACAAAAA																2	
127	ACABAAAAAAAACBA																2	
128	ADDAAACBAAAAAAA												1					
129	AEAAAAAAAAAAAAA												1					
130	AEAAAACAAAAAAAA												1					
131	AEAAAACBBBAAAAA												1					
132	AA/EAAAACBAAAABBA					1												
133	AFAAAAAAAA/BABAAA				1													
134	AGAAAAAAAAABAAA		1															
135	BAAAAABAAAAAAAA/B				1													
136	BAAAAACCAAAAAAA				1													
137	BABAAAAAEAAAAAA			1														
138	BBAAAAAAAEAABAA																	1
139	BBAABACABAAAABA				1													
140	CAAAAAAABBABAAA				1													
141	CAAAAAAACAAABBA																1	
142	CAAAAAABBA/CAAABA				1													
143	CAAAAACAAAAAAAA				1								1					
144	CAAAAAA/CAAAAAAAA				2													
145	CAAAAACAAAAAAAA/B				1													
146	CAAAAACBAAAABBA																1	
147	CAAAAACBAAABBBA																1	
148	CAAAAACBAA/CAAAAA				1													
149	CAAAABCBAAAAAAA																	1
150	DAAAAAAAAAAAAAA		5	8	4											1		
151	DAAAAAAAAAAABAA																1	
152	DAAAAAAABAABAAA						1											
153	DAAAAAAAAABAAAA			2	1													
154	DAAAAAAAAA/CAAAAA				1													
155	DAAAAAAAABAAAAA				1													
156	DAAAAAAABAAAAAA			1	1													
157	DAAAAAAABAABAAA			1	1													
158	DAAAAAA/CBAAAAABA				1													
159	DAAAAAA/CAAA/CAAAAA	1																
160	DAAAAABAAAAAAAA			1														
161	DAAAAABAAAAA/CAAB	1																
162	DAAAAACAAAAAAAA				1													
	Totals	15	40	42	75	10	11	36	24	48	39	24	31	38	12	38	45	26
	Proportion Unique Individuals	–	–	–	0.48	0.31	0.09	0.27	0.04	0.16	0.18	0.41	0.13	0.36	0.00	0.21	0.44	0.35

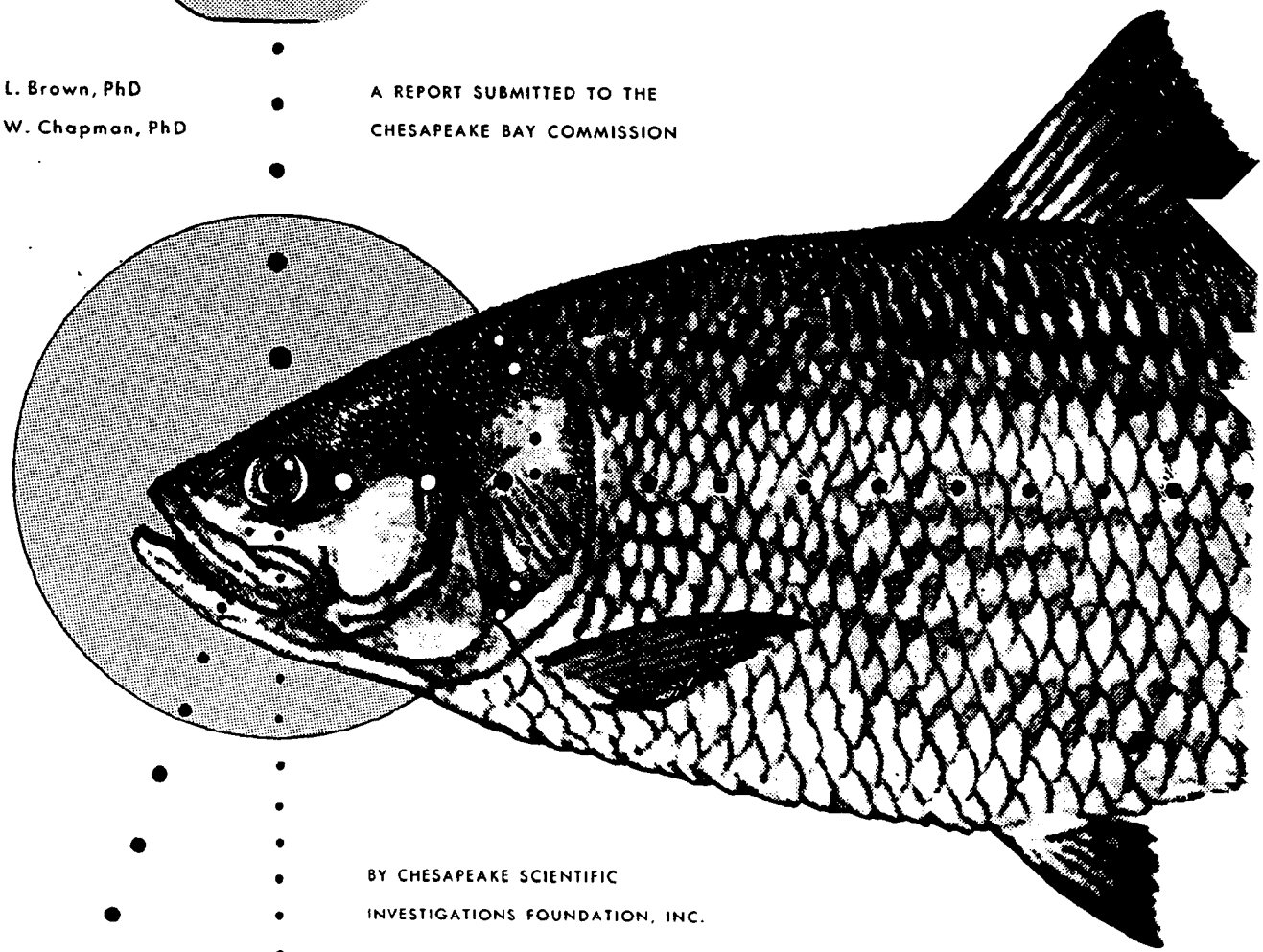
Appendix 17

DRAFT

GENETIC ANALYSIS OF AMERICAN SHAD ENTERING CHESAPEAKE BAY

Bonnie L. Brown, PhD
Robert W. Chapman, PhD

A REPORT SUBMITTED TO THE
CHESAPEAKE BAY COMMISSION



BY CHESAPEAKE SCIENTIFIC
INVESTIGATIONS FOUNDATION, INC.

SEPTEMBER, 1991

GENETIC ANALYSIS OF AMERICAN SHAD ENTERING CHESAPEAKE BAY

Principal Investigators:

Bonnie L. Brown, Ph.D.
Department of Biology
Virginia Commonwealth University
Richmond, VA 23284-2012

Robert W. Chapman, Ph.D.
Department of Biology
East Carolina University
Greenville, NC 27858-4353

A report submitted to:

The Chesapeake Bay Commission
September, 1991

By:

Chesapeake Scientific Investigations Foundation, Inc.

This contracted research was funded by the Pennsylvania Delegation to the Chesapeake Bay Commission, the Virginia Council on the Environment (COE) and the Virginia Marine Resources Commission. COE funding was provided through the Coastal Resources Management Program through grant # NA90AA-H-CZ796 of the National Oceanic and Atmospheric Administration under the Coastal Zone Management Act of 1972 as amended.

ACKNOWLEDGEMENTS

This study was conducted for the Chesapeake Bay Commission. Funds were provided by the Chesapeake Bay Commission, the Virginia Council on the Environment (Coastal Zone Management Grant # NA90AA-H-CZ796), and the Virginia Marine Resources Commission (in-kind services) to perform genetic analysis of American shad, *Alosa sapidissima*, harvested by Virginia watermen from waters outside Chesapeake Bay in Virginia's ocean gill net fishery. Genetic analysis was employed to ascertain, to the maximum extent possible, the natal stream and spawning destination of shad which are susceptible to harvest in the Atlantic Ocean intercept fishery. Genotypes were compared to shad from ten baseline target rivers and from Susquehanna River previously analyzed under MDNR contract number PR90-004-004. Inclusion of Albemarle Sound, Cape Fear and Santee River baseline populations was made possible by a supplemental grant from Atlantic States Marine Fisheries Commission's Interstate Fisheries Management Program (ISFMP) grant number 91-1(PASRH). We thank J. Pella for editing an earlier version of this report and for providing the GIRLSEM algorithm which we used to analyze the genetic data produced by this study. We also thank Barney Jernigan for preparing other essential computer programs and for his assistance with data analysis. A great many other people also contributed to the study by providing biological specimens of American shad including but not limited to:

Walt Ambrogetti, USFWS
Tom Backman, USFWS
Bob Brandt, NY State Dept. of Environmental Conservation
Rick Eager, USFWS
Chris Frese, RMC Environmental Services
Joe Loesch, Virginia Inst. of Marine Science
Art Lupine, NJ Freshwater Fisheries Lab
Ted Meyers, USFWS
Roy Miller, DE Div. of Fisheries & Wildlife
Jim Owens, Virginia Inst. of Marine Science
Fritz Rhode, NC Div. of Marine Fisheries
Dick St. Pierre, USFWS
Mark Tisa, MA Div. Fisheries & Wildlife
Glenn Ulrich, SC Wildlife & Marine Resources Div.
Dale Weinrich, MD Dept. of Natural Resources
Sara Winslow, NC Div. of Marine Fisheries

EXECUTIVE SUMMARY

Genetic analysis was employed to determine the stock composition of American shad, *Alosa sapidissima*, harvested outside Chesapeake Bay in Virginia's Atlantic Ocean intercept fishery during the Spring of 1991. Genotypes of intercept fish were compared to fish from fourteen American shad populations in order to estimate the relative percentage of shad from each baseline population in the intercept sample. Techniques involved restriction endonuclease digestion of mitochondrial DNA (mtDNA) purified from shad egg tissue, a common methodology used for examining population dynamics in fishes. MtDNA genotypes were obtained for 158 individuals from three locations near the mouth of Chesapeake Bay: Rudee Inlet, Chincoteague, and Quinby, VA. The fourteen baseline populations included 585 American shad from spawning aggregations in eleven target rivers (Columbia, Connecticut, Delaware, Hudson, Nanticoke, Pamunkey, James, Chowan, Savannah, St. Johns and Santee Rivers), from the Susquehanna Flats in upper Chesapeake Bay, and from Susquehanna River shad lifted over Conowingo Dam in 1990 and 1991.

Since many shad have mtDNA genotypes which are common to several if not all drainages, every fish cannot be uniquely identified to a river system. Therefore, when a mixed assemblage of fish is examined (such as an ocean fishery), genotypes of the entire group are statistically analyzed for comparison to baseline genetic data for potential source populations. The analysis provides an estimate of the most likely composition of the group in question. The standard statistical treatment which has been developed to interpret mixed-fishery genetic data was created by Dr. J. Pella and co-workers for west coast salmon and has been successfully employed to manage that fishery for many years.

Most baseline American shad populations share one common mtDNA type, but each contains unique types as well. These unique types of mtDNA made it possible to estimate the percentage of each baseline stock represented in the migrating coastal group of shad. Nineteen percent of American shad sampled outside Chesapeake Bay had unique mtDNAs themselves and could not be classified as originating from any of our current baseline populations. The remaining 81% of intercept shad were compared to the baseline populations in a maximum likelihood analysis of stock contribution. Composition of this portion of the intercept group was estimated to be due to contributions of the following six stocks: James (1 ± 13 %), St. Johns (4 ± 4 %), Pamunkey (7 ± 6 %), Santee (7 ± 14 %), Hudson (15 ± 9 %), and Susquehanna (65 ± 12 %). No contribution was detected from the other baseline shad populations. Thus, we estimate that Virginia's 1991 intercept fishery was comprised of approximately 53% Susquehanna shad (0.81 X 65%); perhaps a great deal more if the Hudson and Santee contributions are due to presence of Susquehanna Flats fish in the intercept sample.

These estimates should be considered preliminary for two important reasons. First, the Susquehanna Flats baseline sample is very small and may not adequately represent that group of American shad. Contributions attributed by genetic analysis to Hudson and Santee

Rivers may be due in part to the presence of Susquehanna Flats shad which appear to be of Hudson and Santee River descent. However, the composition of Susquehanna Flats shad is not yet known with certainty due to small sample size from that area. Second, the Susquehanna River population does not appear to have attained genetic stability and it is possible that contribution estimates for this River will vary from year to year as the resurgent Susquehanna population approaches a stable genetic equilibrium.

Despite small sample sizes, a trend is evident in the genetic composition of shad harvested north of the Bay mouth (Chincoteague and Quinby) and those harvested south of the Bay mouth (Rudee). Seventy percent of Rudee Inlet shad were of Virginia origin while the Chincoteague and Quinby harvests were composed primarily of Susquehanna, Hudson and Santee shad.

To consider the potential effect of the coastal intercept fishery we would need several years of estimates of the stock composition and magnitude of both Maryland and Virginia's coastal harvests. However, a rough estimate can be made based on Virginia's preliminary estimate of their 1991 ocean shad harvest and the present genetic data. The estimated amount of shad harvested in 1991 was 405,612 pounds. Multiplying by a factor of 0.53, approximately 215,000 pounds were of Susquehanna origin. This is roughly twice the poundage of shad lifted over Conowingo Dam in the Spring of 1991. If these findings are verified by future monitoring, tagging and genetic evaluation then the most conservative action would be to restrict shad harvests along the Delmarva Peninsula.

INTRODUCTION

The American shad, *Alosa sapidissima*, is an anadromous member of the herring family (Clupeidae), which ranges from the Gulf of St. Lawrence to Florida (Walburg and Nichols, 1967). During its springtime spawning runs the species has been subjected to substantial commercial and recreational fishing pressure throughout its range, particularly in Chesapeake Bay tributaries and by Maryland and Virginia's ocean fisheries. In addition, shad populations of almost every Chesapeake Bay drainage have been further restricted by dams which block migration to their spawning habitat in fresh water transition zones. As a result of fishery exploitation, loss of spawning and nursery habitat, and possibly environmental degradation such as stream acidification, harvests of shad in Pennsylvania, Maryland, the District of Columbia and Virginia declined precipitously during the period 1965-1988 (Stagg, 1986; Gibson, Crecco and Stang, 1988).

A great deal of effort has been expended to revive stocks of Chesapeake Bay American shad. Conservation and restoration measures were enacted in Pennsylvania, Maryland and the District of Columbia in the early 1980s. Some agencies required season, gear and by-catch restrictions along with creel limits to reduce fishing effort (Maryland's shad fishery was closed in 1980). Concurrently, the issues of habitat loss and degradation were addressed by installing permanent fish passage facilities such as the one at Conowingo Dam, removing some obstructions to migrating fish, re-stocking fish into historical spawning habitats, establishing stock assessment and monitoring programs, and operating dam turbines in a manner which maintained minimum flow and standard dissolved oxygen levels (CEC, 1989).

Prior to these efforts, American shad migrating upstream in Susquehanna River had been few in number. Throughout the 1970s shad transported by the trap/lift assembly at Conowingo Dam averaged 127 fish per year (ASMFC, 1988). Yearly release of shad fry and of live pre-spawned adult shad from six other source rivers accompanied the lift operation beginning in 1982 (Table 1). By 1989, more than 6000 migrating shad were reported to have been hauled upstream above all dams to the historical Susquehanna spawning areas.

In view of the apparent success of shad management efforts in Susquehanna River, a program of study was proposed to the Maryland DNR Chesapeake Bay Research and Monitoring Division's Power Plant Topical Research Program designed to examine population dynamics underlying the resurgent American shad population in Susquehanna River using molecular genetic techniques (Chapman and Brown, 1991). Mitochondrial DNA genotypes of American shad being moved over Conowingo Dam were compared to genotypes of shad from the source rivers, from other Chesapeake Bay rivers and from several southern east coast shad populations. Variation in mitochondrial DNA was analyzed and employed to estimate the percent contribution by any of these shad populations to the increasing Susquehanna stock.

Population genetic data collected for the Maryland DNR study were employed in the present study for Chesapeake Bay Commission to estimate the relative percentage of American shad from each baseline population being harvested in Virginia's Atlantic Ocean intercept fishery during the Spring of 1991. The baseline populations available for comparison with the Spring 1991 coastal fishery were: Columbia River-WA, Delaware River-DE, Hudson River-NY, Connecticut River-CT, Nanticoke River-MD, Pamunkey River-VA, James River-VA, Chowan River-NC, Savannah River-SC, Santee River-SC and St. Johns River-FL, the Susquehanna Flats in upper Chesapeake Bay, and Susquehanna River shad lifted over Conowingo Dam in 1990 and 1991. This report summarizes the seven-month research project, provides estimates of proportions of American shad from each of the groups outlined above which comprise the Spring 1991 coastal harvest, and outlines management and research implications of these data.

LABORATORY ANALYSIS

The laboratory procedures described in this report are intentionally brief. Detailed instructions for the extraction and digestion of mitochondrial DNA can be found in Chapman and Brown (1990).

During the Spring of 1991, mitochondrial DNA (mtDNA) was extracted from American shad harvested in Virginia's Atlantic Ocean intercept fishery outside the mouth of Chesapeake Bay. Shad were obtained from commercial fishermen landing at three locations: Rudee Inlet, Chincoteague and Quinby/Wachapreague (see Figure 1). Ovaries from each individual fish were removed and placed in a Ziplock Baggie along with an envelope containing a scale sample and pertinent data on the location of capture, size of fish, etc. Each baggie was sealed and placed on wet ice for transportation to the laboratory at East Carolina University in Greenville, NC.

All shad samples arrived at the laboratory on the day they were collected. Within one day of sampling, approximately 10 g of egg tissue from each individual fish were processed to isolate purify mtDNA. The mtDNA was rehydrated in 150 µl of sterile distilled water and aliquots of 8.5 µl mtDNA from each fish were combined with 0.5 Unit of the following restriction endonucleases (*Aat* I, *Apa* I, *Bcl* I, *Bgl* I, *Dra* I, *Eco*R I, *Eco*R V, *Hind* III, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, *Sma* I, *Sst* II, and *Xba* I) along with 1 µl of the appropriate buffer supplied by the manufacturer. Each digest was incubated at 37 ^{o}C for 3-4 hours and contained a total volume of 10 µl. Reactions were stopped with 1 ul of STOP solution (0.89 M Tris, 0.89 M boric acid, 0.02 M EDTA, 0.25 % bromophenol blue, 50 % glycerol and 1 % SDS) and were electrophoresed overnight through 0.8 % agarose gels. The DNA in gels was stained with ethidium bromide and photographed under ultra-violet light as described by Chapman and Powers (1984).

Restriction digest patterns were recorded for each restriction endonuclease digest of each fish's mtDNA. Digestion patterns were assigned upper-case alphabetic symbols. Then, each individual was assigned a composite "haplotype" consisting of the letters designating the restriction fragment patterns produced by digestion with each of the fifteen enzymes.

DATA ANALYSIS

Details of the mathematical properties of the algorithms used to perform statistical analyses can be found in Sokal and Rohlf (1981), Roff and Bentzen (1989), Fournier *et al.* (1984), Pella (1986), and Pella and Milner (1987).

Each fish's haplotype is a multiple characterization of that fish's mitochondrial genome and is transmitted in a manner analagous to human surnames. As in other animals, variation in shad mtDNA is typified by the occurrence of rare haplotypes in each population (Bentzen *et al.*, 1988 and 1989). If chi-square contingency tests were to be performed the rare haplotypes would be lumped and only the most frequent haplotypes would be employed in the analysis. This practice of pooling rare mtDNA haplotypes results in a severe loss of information relevant to geographic and temporal genetic variation. To resolve this problem, Roff and Bentzen (1989) presented a chi-square analysis which does not require pooling of rare variants. The analysis generates Monte Carlo distributions of expected chi-square from unpooled mtDNA data and allows high levels of significance even when sample sizes are small.

Haplotype frequencies were employed to determine basic genetic relationships between the baseline shad populations and the intercept population. First, a series of chi-square statistics for heterogeneity of mtDNA haplotype frequencies were calculated (Roff and Bentzen, 1989). The chi-square analysis was conducted by treating all of the populations as one large assemblage. Then, based on the finding of significant heterogeneity, successively smaller sets of populations were analyzed until no further heterogeneity was detected.

The actual contribution of each baseline population to the intercept sample was estimated from the recorded mtDNA information by the method of conditional maximum likelihood estimation of stock composition as proposed by Fournier *et al.* (1984) and by Ferris and Berg (1987). The basic concepts underlying this genetic analysis are similar to a traditional mark-recapture study. The algorithm we used was created by J. Pella of NMFS (Pella, 1986) for interpretation of mixed-fishery genetic data for west coast salmon. Called GIRLSEM, the computer program written in FORTRAN is an iteratively reweighted least squares algorithm to compute the conditional maximum likelihood estimate of composition of a group of fish of mixed ancestry. An additional benefit of this program is that sampling error, which leads to variability of the composition estimates, can be assessed by bootstrap resampling and by the infinitesimal jackknife procedure. The accuracy of this method was explored by analyzing several artificial populations of known composition created by sampling the baseline haplotypes (with replacement) prior to the analysis of mixed shad populations.

RESULTS

A total of 162 individual shad collected during a one-month period from three locations near the mouth of Chesapeake Bay were processed. Fifteen restriction endonuclease digests were performed per individual for a total of 2,430 digests. Thirty-three restriction fragment profiles were observed in the intercept samples, encompassing seventy-eight separate restriction fragments of the *Alosa sapidissima* mitochondrial genome. Whenever possible the profiles for an enzyme were compared to those obtained by Bentzen *et al.* (1988 and 1989). Restriction fragment profiles for each enzyme are illustrated in Fig. 2.

Genotypes for each individual intercept shad are shown in Appendix A. Haplotypes, the composites of all fifteen genotypes, for each intercept shad are shown in Table 2 and are also listed in Appendix B alongside haplotypes for all of the baseline populations. The frequencies of each haplotype for each of the three locations examined are shown in Table 2 along with the frequencies of haplotypes in each of the baseline populations. Thirty-four different haplotypes were detected in the intercept samples. Seventy-nine shad had either the common haplotype AAAAAAAAAAAAAAA or one of the many unique baseline haplotypes. Haplotypes of eighteen individuals in the intercept sample were unique. In some instances the mtDNA preparation was of inadequate quantity or quality to perform all fifteen digests. Sixty-five shad were incompletely characterized and were not included in the final analysis (missing data are shown by "-" in Appendix A).

Results of a chi-square analysis based on the distribution of haplotypes are presented in Table 3. The first tier of the chi-square analysis was performed with the entire sample (baseline and intercept) as one assemblage (overall chi-square of 4765.41, $P < 0.0001$) and indicated that significant differences existed within the aggregation. The assemblage was successively decomposed by population to the point where no further heterogeneity was detected among the groups shown in Table 3. An important finding of this analysis was that shad harvested from Rudee Inlet were significantly different from those taken at Chincoteague and Quinby (chi-square of 94.78, $P < 0.001$). Also, no significant difference was detected between the Chincoteague and Quinby samples (chi-square of 33.78, $P = .717$).

Prior to the final analyses, the GIRLSEM program was tested by compiling several subsets of known composition from the baseline haplotypes in order to evaluate accuracy and precision when estimating the composition of mixed stock samples from shad mtDNA haplotypes (Chapman and Brown, 1991). Results of these analyses are shown in Table 4. GIRLSEM analysis performed well under the artificial scenarios and accurately estimated the actual contribution of baseline populations in most instances. Estimates were statistically acceptable and required fewer than 100 iterations to converge. Instances where estimates were incorrect could be attributed to contributions by baseline populations which had a very low portion of unique individuals. One river in the present baseline data set, Connecticut River, falls into this category, where only one of 24 fish sampled was unique. In the trials, actual contribution by Connecticut was consistently attributed to Delaware River.

Incompletely characterized shad and the nineteen percent of intercept shad whose haplotypes were unique (not observed in any baseline population) were withheld from the final analysis. Haplotypes for the remaining intercept samples were compared to those of all baseline populations. Every haplotype which was shared between the intercept sample and any baseline population was used in final runs to estimate percent composition of the intercept sample. The number of intercept shad employed in this analysis was seventy-nine (81% of the total intercept sample). Separate conditional maximum likelihood analyses were also conducted for shad from each of the three sample locations. Table 5 lists the estimated composition of the 1991 Virginia intercept shad fishery. By multiplying the maximum likelihood results in Table 5 by a factor of 0.81, it is estimated that the following populations comprised the 1991 intercept harvest: Unknown (19.37%), James (0.81%), St. Johns (3.26%), Pamunkey (5.70%), Santee (5.70%), Hudson (12.22%), and Susquehanna (52.94%). These estimates are depicted graphically in Figure 3.

DISCUSSION

American shad examined for this study and for the larger MDNR study are of diverse genetic composition. In order to evaluate composition of the intercept fishery it was first necessary to evaluate composition of Susquehanna and other Chesapeake Bay stocks. Chapman and Brown (1991) reported that the existing Susquehanna River stock is comprised of shad descended from both native Chesapeake Bay and southern Atlantic coast rivers and that the Susquehanna stock does not appear to be in a state of genetic equilibrium.

Chi-square analysis indicated American shad landed at different Atlantic Ocean locations were significantly different. Those landed at Rudee Inlet were different from shad landed along the Virginia portion of the Delmarva Peninsula. Shad harvested along the Delmarva Peninsula by the intercept fishery (Chincoteague and Quinby) were not significantly different from one another.

Nineteen percent of the intercept fish could not be classified by our current baseline data set. This indicates that the baseline data set must be expanded before this portion of the intercept fishery can be accurately evaluated. Conditional maximum likelihood estimates of the composition of the remaining 81% of the intercept fishery sample indicate that those intercept shad were predominantly of Susquehanna and Hudson River origin (Table 5). Small contributions by other locations, from both Chesapeake Bay and southern rivers, were also detected in the overall analysis. Although the standard deviation for the largest contributing population, Susquehanna, is marginally acceptable, other standard deviation values are large (Table 5). This situation occurs due to small sample sizes.

Small sample sizes made it difficult to estimate the probable destination of shad landed at the Rudee, Chincoteague and Quinby locations separately with an acceptable degree of confidence. The final sample sizes for Rudee, Chincoteague and Quinby are small (n = 15, 40, 42, respectively) after excluding partial and unique haplotypes (17%, 7%, and 9%, respectively) and the standard deviation values for these estimates are proportionally large (see Table 5). However, a general trend is apparent. Most shad analyzed from Rudee Inlet were estimated to be of Virginia origin (70% Pamunkey and James, 30% Susquehanna) while the Chincoteague and Quinby harvests were composed of Susquehanna, Hudson and Santee shad.

CONCLUSIONS

This project involved a genetic survey to identify which, if any, target populations were harvested by Virginia's Atlantic Coast intercept fishery. The study was conducted concurrently with one of the most comprehensive investigations of a mixed fishery attempted to date employing mtDNA analysis. The results presented here are of immediate short-term use to the community of managers and scientists who regulate the American shad fishery in Chesapeake Bay. Furthermore, these data constitute a minimum framework which when expanded will allow development of a long-term monitoring program which could eventually rival that for west coast salmon in its effectiveness.

The genetic analysis provides an intitial "snapshot" of Virginia's intercept fishery indicating that at least one-half of the shad harvested were destined for Susquehanna River. Due to the magnitude of effort and funds expended by all of the Bay states to rebuild shad stocks, policy makers and managers are sure to inquire: "Does the Virginia ocean shad harvest potentially affect the Susquehanna stock?" This question can be addressed by considering the preliminary estimate of shad harvested by Virginia's ocean fishery in 1991 (405,612 lb) multiplied by the proportion of those harvested which were estimated to be of Susquehanna origin (53 %) and comparing the figure to Susquehanna shad abundance in 1991 (22,083 shad were lifted over Conowingo Dam @ 4.5 lb = 99,374 lb). Thus the Virginia 1991 harvest may be twice as great as the quantity of Susquehanna shad moved over Conowingo Dam. Seventy-eight percent of Virginia's ocean shad harvest occurs north of the Bay mouth. Based on these values and the genetic results from Rudee Inlet vs the Peninsula locations, the impact on the Susquehanna population could be greatly diminished by restricting harvests along the Peninsula.

This raises a very important issue. We know that Maryland's ocean shad catch increased from 40,000 lb in 1980 to 143,300 lb in 1988 (Flagg, 1990). It would be useful from both management and policy standpoints to estimate the composition of that fishery as well.

The joint MD/VA shad tagging study at Rudee Inlet has produced some preliminary results which should be compared with the genetic data. The two studies are complementary in a very important way. Fifty-six percent of tag returns during the last six months have been from lower Chesapeake Bay; primarily from York River and its tributaries (Jesien and Hocutt, 1991). By comparison the genetic analysis estimates that 66 % of the Rudee Inlet harvest is of American shad from Pamunkey River (a tributary of York River). Such close agreement would tend to indicate that the genetic estimates of stock contribution for this location are correct.

Another aspect of how the tagging and genetic analyses complement one another is associated with the fact that no tags have been returned from upper Chesapeake Bay. There is no fishing effort for shad in upper Chesapeake Bay since both MD and PA have closed their

shad fisheries. Yet the genetic analysis of the ocean catch provides the upper Bay component (Susquehanna River) in addition to the Pamunkey and James components.

In the future, tagging could address some critical issues raised by the genetic analysis. For example, both the Susquehanna Flats and the Susquehanna River shad populations have large Santee River components. It cannot be determined from the present data whether the Santee component of the intercept fishery is due to shad actually returning to Santee River or whether it is due to Susquehanna shad with Santee haplotypes. If tagging endeavors are repeated once fishing resumes in upper Chesapeake Bay then we can evaluate both possibilities.

Like most other scientific investigations the present genetic analysis raises as many questions as it answers. Although the baseline data were adequate to address the majority of intercept fish harvested in 1991, they must be expanded before the entire fishery can be evaluated. We recommend increasing all baseline sample sizes to at least 50 and assessing existing Chesapeake Bay shad populations which were not examined in the present study (Rappahannock, Potomac, Patuxent, Choptank, Chester, etc.). In addition, it is clear that one sample is not adequate to formulate far-reaching management decisions. Shaklee *et al.* (1990) have examined mixed-stock fisheries of Pacific salmon. They found that stock composition varies substantially from year-to-year for mixed-stock assemblages. It would be prudent to assume that migratory mixtures of American shad stocks behave in a similar manner to Pacific coast salmonid stocks.

Depending on the Bay States' goals, we can suggest the necessary actions to undertake. First, if the sole purpose is to make an immediate management decision regarding coastal intercept shad fisheries then at least one more survey should be made of the Virginia fishery accompanied by at least two years of investigation of Maryland's ocean shad fishery. Analyses should proceed by sacrificing 150 fish per site per year, extracting mtDNA, and digesting mtDNA with the same 15 enzymes employed in the present study. The resulting haplotypes should be compared to an expanded data base. This should provide adequate information to confidently make decisions pertaining to the intercept fishery.

If goals are broader and the States wish not only to manage and regulate the intercept fisheries but to monitor all Chesapeake Bay shad populations, then we would suggest a modified research/monitoring program. This program involves conducting the research described above to address the immediate issue of the impact of the intercept fisheries. However, we would also recommend converting previously collected samples to a new format (PCR) which would allow all subsequent genetic analyses to be made from material obtained by amplifying mtDNA from non-lethal biopsy tissue samples (an obvious benefit when genetic analysis is associated with tagging). Tissue samples could be archived and analyzed at any time in the future.

Shad populations are dynamic entities influenced by both natural and anthropomorphic factors. Genetic analysis offers a means not just to estimate the percent composition of mixed assemblages of shad but also to monitor the success of stocks as they respond to ecological changes and to various management practices. Like tagging, annual collection of catch-effort statistics, and other management programs, a program of genetic population analysis requires long-term commitment at the very least to collect and archive samples. Most biological monitoring programs require a minimum of four years of sequential monitoring before any type of trend can be determined. After that, analyses can be performed annually or bi-annually to re-evaluate important groups of Chesapeake Bay and intercept shad.

SIGNIFICANT POINTS

*Virginia's 1991 intercept fishery was comprised of approximately 53% Susquehanna shad (0.81 X 65%), perhaps more.

*This estimate should be considered preliminary for two important reasons. First, the Susquehanna River population does not appear to have attained genetic stability and it is possible that contribution estimates for this River will vary from year to year. Second, the Susquehanna Flats baseline sample is very small and may not completely represent that group of American shad.

*Genetic analysis indicates that the Rudee Inlet harvest differs from harvests along Virginia's portion of the Delmarva Peninsula.

*These findings should be verified by a joint MD/VA investigation which would expand the current genetic baseline data set, identify specific river stocks within both states' intercept fisheries, and establish a long-term shad monitoring program (associated with joint tagging efforts).

REFERENCES

Altukhov, Y.P. and E.A. Salmenkova. 1987. Stock transfer relative to natural organization, management, and conservation of fish populations. Chapter 14 *In*: Population genetics and fishery management. N. Ryman and F. Utter, eds. Univ. of Washington Press, Seattle.

ASMFC. 1988. 1988 Supplement to American shad and river herrings fishery management plan. Fisheries Management Rept. No. 12 of the Atlantic States Marine Fisheries Commission. October 1988, Washington, DC.

ASMFC. 1990. 1990 Shad and river herring workshop, October 16-17, 1990, Nags Head, NC. Atlantic States Marine Fisheries Commission, Washington, DC.

Avise, J.C., J.E. Neigel and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. 20: 99-105.

Backman, T. National Fisheries Research and Development Laboratory, USFWS, Wellsboro, PA.

Bentzen, P., W.C. Leggett and G.G. Brown. 1988. Length and restriction site heteroplasmy in the mitochondrial DNA of American shad (*Alosa sapidissima*). Genetics 118: 509-518.

Bentzen, P., G.G. Brown and W. C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). Can. J. Aquat. Sci. 46: 1446-1454.

Birky, C.W., T. Maruyama and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103: 513-527.

Brown, B.L. and K.T. Paynter. 1991. Mitochondrial DNA analysis of native and selectively inbred Chesapeake Bay oysters, *Crassostrea virginica.* Marine Biology (in press).

CEC. 1989. Chesapeake Bay Alosid Management Plan. An agreement commitment report from the Chesapeake Executive Council. July 1989, Annapolis, MD.

Chapman, R.W. and B.L. Brown. 1990. Mitochondrial DNA isolation methods. Chapter 4 *In*: Electrophoretic and isoelectric focusing techniques in fisheries management. D.H. Whitmore, ed. CRC Press, Boca Raton, FL.

Chapman, R.W. and B.L. Brown. 1991. Genetic investigation of the resurgence of American shad, *Alosa sapidissima*, in Susquehanna River. Report submitted to Maryland Dep. of Natural Resources, Chesapeake Bay Research and Monitoring Division, Annapolis, MD.

Chapman, R.W. and D.A. Powers. 1984. A method for rapid isolation of mitochondrial DNA from fishes. Maryland Sea Grant Tech. Rept. No. UM-SG-TS-84-05.

Dadswell, M.J., G.D. Melvin and P.J. Williams. 1983. Effect of turbidity on the temporal and spatial utilization of the inner Bay of Fundy by American shad (*Alosa sapidissima*)(Pisces:Clupeidae) and its relationship to local fisheries. Can. J. Fish. and Aquat. Sci. 40 (Suppl. 1): 322-330.

Ferris, S.D. and W.J. Berg. 1987. The utility of mitochondrial DNA in fish genetics and fishery management. Chapter 11 *In*: Population genetics and fishery management. N. Ryman and F. Utter, eds. Univ. of Washington Press, Seattle.

Flagg, L. 1990. Review of Atlantic States Marine Fisheries Commission Fishery Management Plan for American shad and river herring. pp 76-82 *In*: Atlantic States Marine Fisheries Commission 1990 shad and river herring workshop. Oct. 16-17, 1990, Nags Head, NC.

Fournier, D.A., T.D. Beacham, B.E. Riddell and C.A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. and Aquat. Sci. 41: 400-408.

Gibson, M., V. Crecco an dD. Stang. 1988. Stock assessment of American shad from selected Atlantic coast rivers. Special Report No. 15 of the Atlantic States Marine Fisheries Commission, Washington, DC.

Gibson, M. Rhode Island Div. of Fish and Wildlife, W. Kingston, RI.

Jesien, R. and C. Hocutt. 1991. Movement of American shad in the Chesapeake Bay. Progress Report to MDNR, Annapolis, MD.

Leggett, W.C. and R.R. Whitney. 1972. Water temperature and the migration of American shad. Fish. Bull. (US) 70:659-670.

Melvin, G.D., J.D. Michael and J.D. Martin. 1985. Fidelity of American shad, *Alosa sapidissima*, (Osteichthyes:Clupeidae) to its river of previous spawning. Can. J. Fish. and Aquat Sci. 42:1-19.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Pella, J.J. 1986. The method of fitting expectations applied to computation of conditional maximum likelihood estimates of stock composition from genetic marks. US DOC/NOAA/NMFS/NWAFC Auke Bay Laboratory, Auke Bay, AK. Rept. No. 171.

Pella, J.J. and G.B. Milner. 1987. Use of genetic marks in stock composition analysis. Chapter 10 *In*: Population genetics and fishery management. N. Ryman and F. Utter, eds. Univ. of Washington Press, Seattle.

Roff, D.A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: Chi-square and the problem of small samples. Mol. Biol. Evol. 6: 539-545.

Shaklee, J. C. Busack, A. Marshall, M. Miller and S. Phelps. 1990. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon. pp. 235-265 *In*: (Z-I. Ogita and C. Marke, eds.) Isozymes: Structure, Function, and Use in Biology and Medicine. Prog. in Clin. and Biol. Res. Vol. 344. Wiley-Liss, Inc. New York.

Sokal, R.R. and F. J. Rohlf. 1981. Biometry. W.H. Freeman and Co., San Francisco.

Stagg, C. 1986. An evaluation of the information available for managing Chesapeake Bay fisheries: preliminary stock assessments, Vol. I and II. Univ. Maryland, CBL, UMCEES [CBL] Ref. No. 85-29.1.

Takahata, N. and T. Maruyama. 1981. A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res., Camb. 37: 291-302.

Walburg, C.H. and R.P. Nichols. 1967. Biology and management of the American shad and status of the fisheries. Atlantic coast of the United States 1960. US Fish and Wildl. Ser. Special Sci. Rept. Fish. No. 550.

SUGGESTED PEER-REVIEWERS

Richard St. Pierre
Susquehanna River Coordinator
USFWS
1721 N. Front Street, Suite 105
Harrisburg, PA 17102

James Shaklee, Ph.D.
Washington Dept of Fisheries
115 General Administration Bldg.
Olympia, WA 98504

John Graves, Ph.D.
Virginia Institute of Marine Science
College of william and Mary
Gloucester Point, VA 23062

Paul Bentzen, Ph.D.
Dept. of Biology
Dalousie University
Halifax, Nova Scotia
CANADA B3H 4J1

Figure 1. Map of Virginia's Atlantic coastline showing locations of the three landing areas sampled for American shad: Rudee Inlet, south of the Bay mouth, Quinby and Chincoteague, north of the Bay mouth.

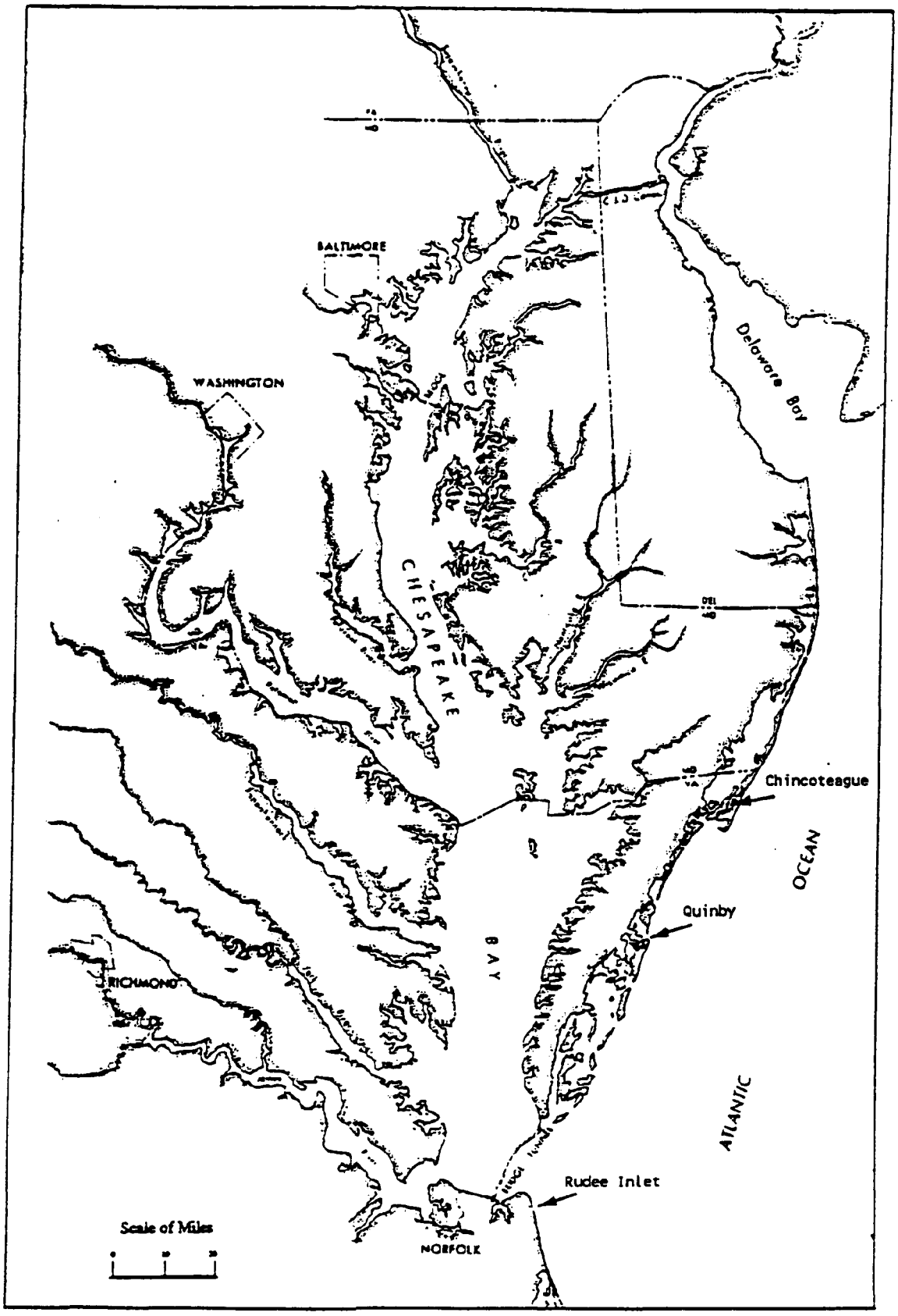
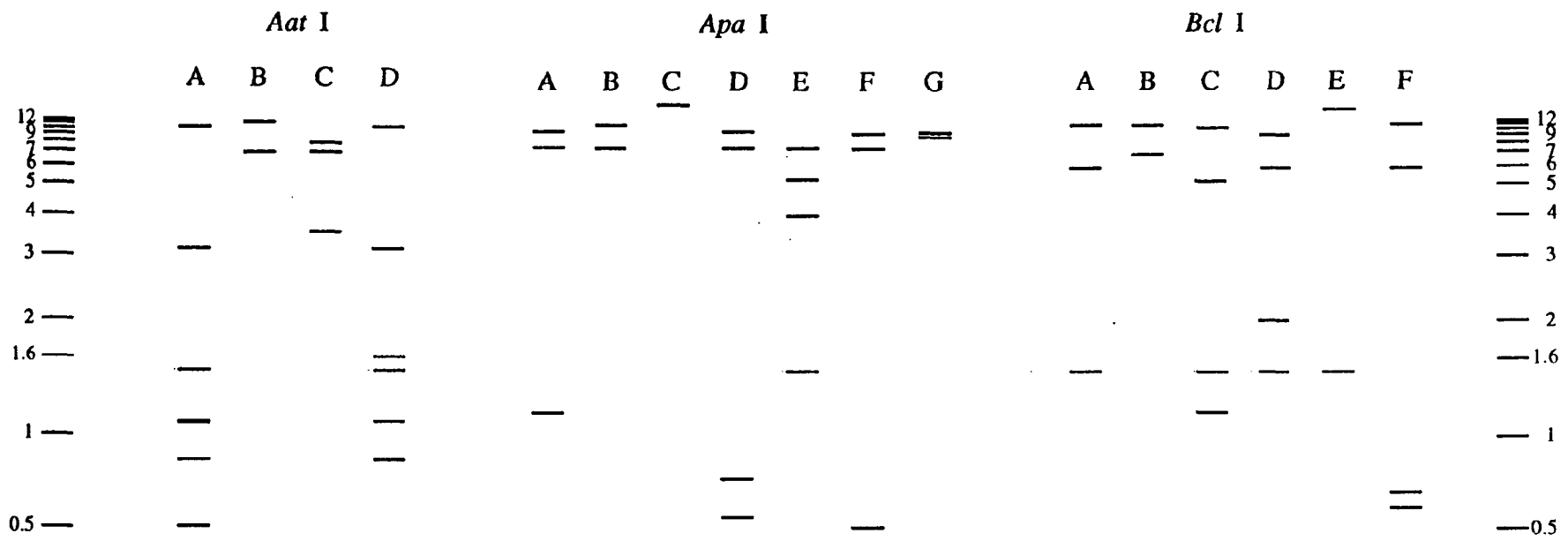
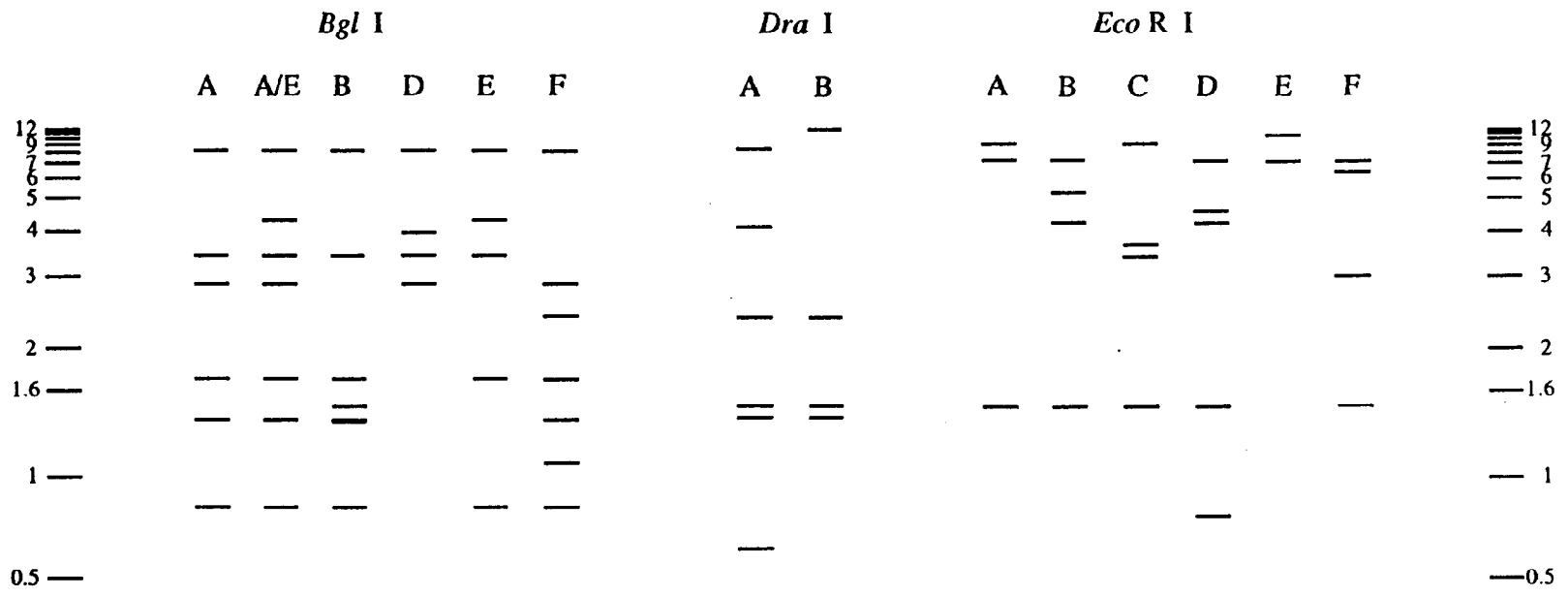
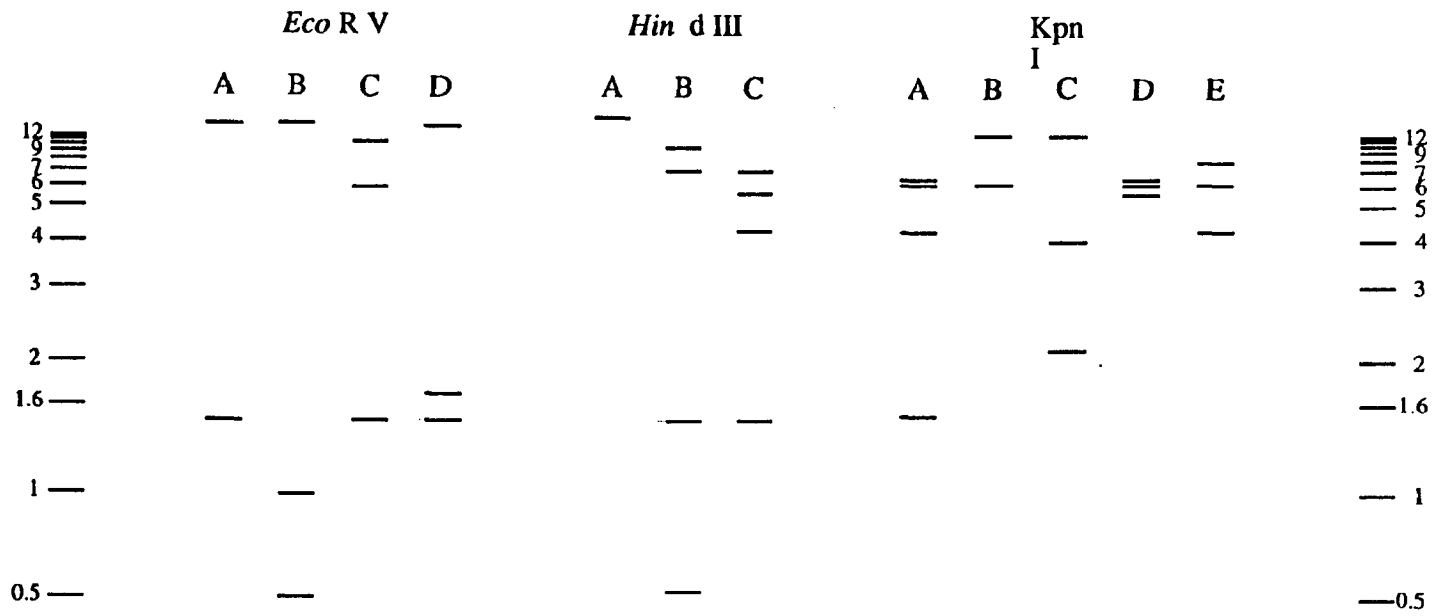
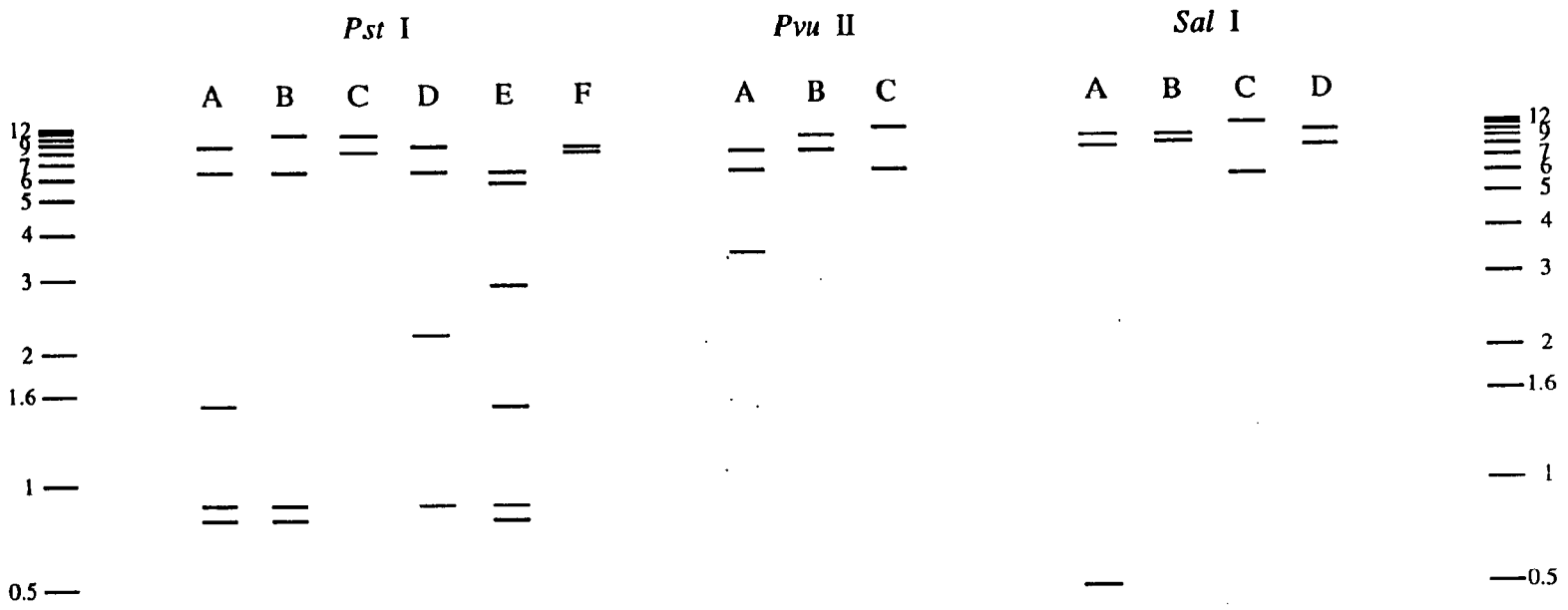

BALTIMORE
WASHINGTON
Delaware Bay
CHESAPEAKE
BAY
RICHMOND
Chincoteague
OCEAN
Quinby
ATLANTIC
Rudee Inlet
NORFOLK
Scale of Miles

Figure 2. Graphic representation of American shad restriction fragment patterns. Names of restriction enzymes occur along the top margin and are those listed in the text. Genotype names assigned to each pattern are the capitol letters directly underneath each enzyme. Bands of a molecular weight standard are shown along the left and right margins of each page and the sizes of these bands are indicated in kilobases (thousands of nucleotide base pairs).


Aat I
A
B
C
D
Apa I
A
B
C
D
E
F
G
Bcl I
A
B
C
D
E
F
12
9
7
6
5
4
3
2
1.6
1
0.5


Bgl I
A
A/E
B
D
E
F
Dra I
A
B
Eco R I
A
B
C
D
E
F
12
9
7
6
5
4
3
2
1.6
1
0.5


Eco R V
Hin d III
Kpn I
A
B
C
D
A
B
C
A
B
C
D
E
12
9
7
6
5
4
3
2
1.6
1
0.5


Pst I
A
B
C
D
E
F
Pvu II
A
B
C
Sal I
A
B
C
D
12
9
7
6
5
4
3
2
1.6
1
0.5

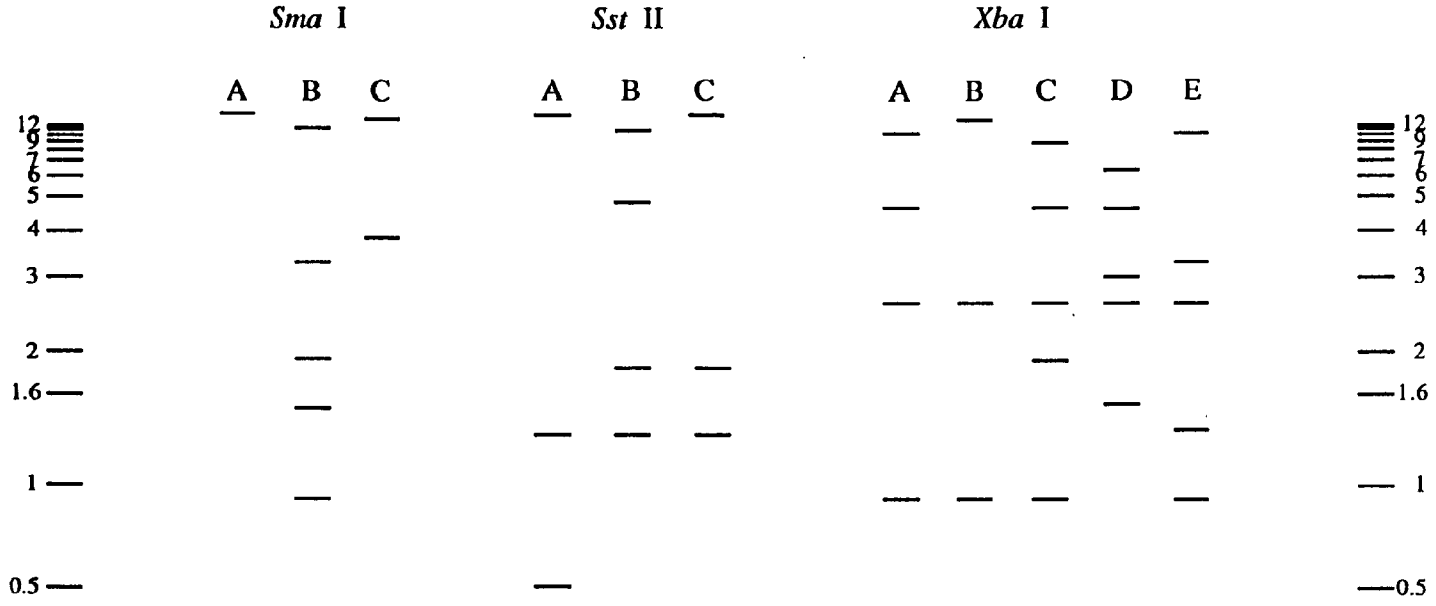

Sma I
A
B
C
Sst II
A
B
C
Xba I
A
B
C
D
E
12
9
7
6
5
4
3
2
1.6
1
0.5

Figure 3. Graphic illustrations of the percent composition of the 1991 ocean shad harvest. A. Total catch, B. Rudee Inlet, C. Chincoteague and D. Quinby.

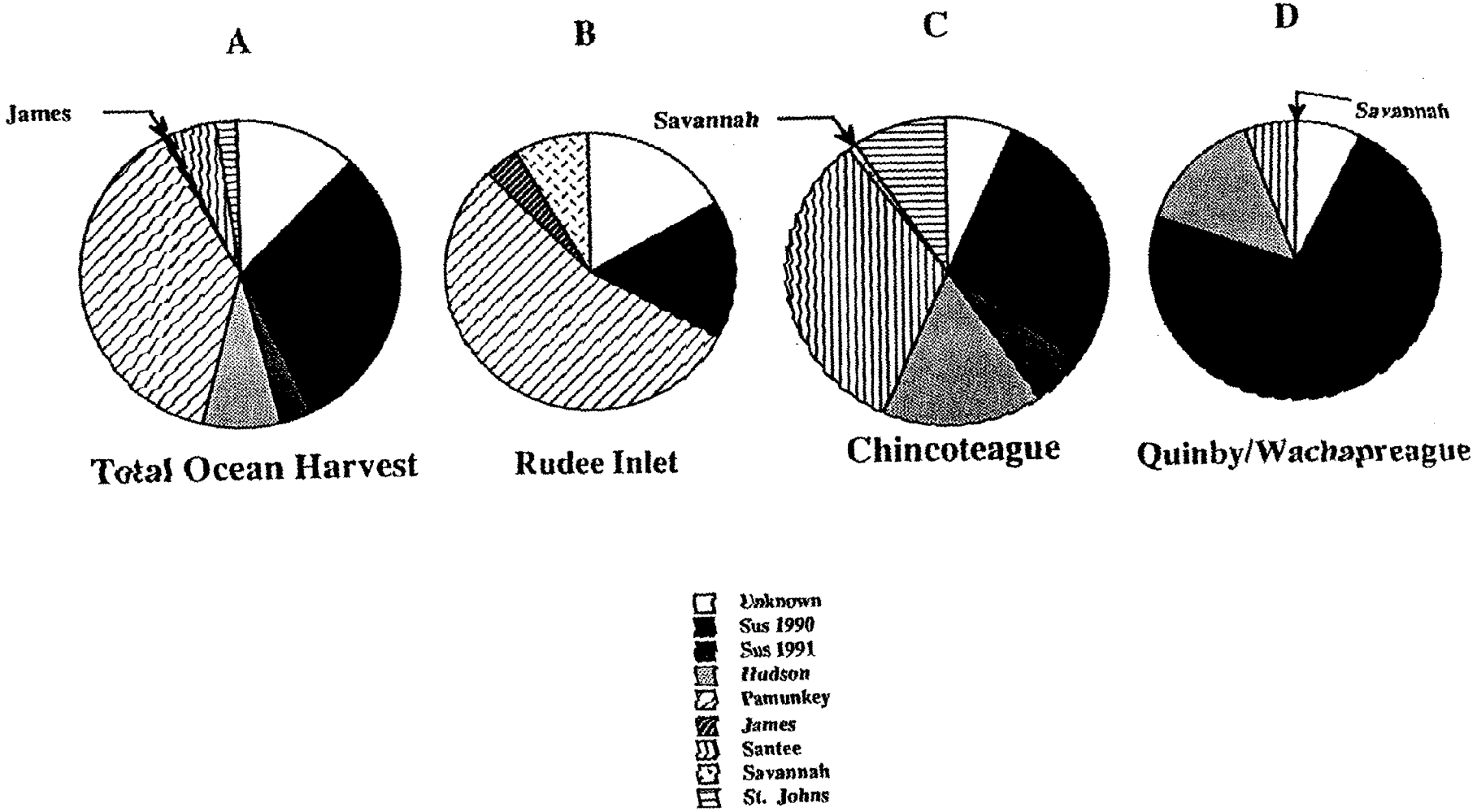

A
B
C
D
James
Savannah
Savannah
Total Ocean Harvest
Rudee Inlet
Chincoteague
Quinby/Wachapreague
Unknown
Sus 1990
Sus 1991
Hudson
Pamunkey
James
Santee
Savannah
St. Johns

Table 1. Summary of American shad stocked into the Susquehanna River during the period 1982-1991 by river of origin

A. Hatchery cultured fry (millions)

Year	Pamunkey	James	Columbia	Delaware	Hudson
1991	-	-	-	3.212	8.845
1990	0.178	-	-	3.565	6.000
1989	0.754	0.220	12.422	1.645	5.660
1988	0.655	0.029	8.467	0.949	-
1987	1.403	0.040	6.919	1.227	-
1986	2.433	0.210	11.184	1.243	-
1985	2.222	0.458	1.906	1.642	-
1984	4.289	0.509	7.162	0.380	-
1983	1.000	1.100	1.950	-	-
1982	1.151	0.740	6.949	-	-

B. Live pre-spawned adults

Year	Susquehanna	Hudson	Connecticut
1991	22083	-	-
1990	14792	-	-
1989	6590	-	-
1988	4730	-	-
1987	6900	6032	-
1986	4080	4965	-
1985	950	3158	64
1984	0	3592	185
1983	0	3123	1187
1982	875	992	1573

Table 2. Mitochondrial DNA haplotypes of American shad harvested outside Chesapeake Bay in 1991.

Haplotype	Rudee	Chincoteague	Quinby
AAAAAAAAAAAAAAA	2	16	14
AAAAAAAAAAAAAAA/B	0	1	0
AAAAAAAAAAAAAAB	0	1	0
AAAAAAAAAAABAAA	0	1	1
AAAAAAAAAABAAAA	0	1	1
AAAAAAAAA/BAAA/BAAA	0	0	1
AAAAAAAABAAAAAA	1	4	5
AAAAAAAABAAAAAB	1	0	0
AAAAAAAABAAAABA	1	0	0
AAAAAAAABAABAAA	1	7	3
AAAAAAAABEAAAAA	1	0	0
AAAAAAAACAAAAAA	0	0	1
AAAAAABABAAAAAA	0	0	1
AAAAAABABAABAAA	0	0	1
AAAAAACAAAAA/CAAB	1	0	0
AAAAAACAAAACAAA	1	0	0
AAAAAACABAAAAAA	1	0	0
AAAAAACABAAAAAB	1	0	0
AAAAAACABAAA/CAAB	1	0	0
AAAAABAAAAAAAAA	0	1	0
AAAABAAAAAAAAAA	0	1	0
AAAFAAAAAAAAAAA	1	0	0
ABAAAAAAAAAAAAA	0	1	0
AGAAAAAAAAABAAA	0	1	0
BABAAAAAEAAAAAA	0	0	1
DAAAAAAAAAAAAAA	0	5	8
DAAAAAAAAABAAAA	0	0	2
DAAAAAAABAAAAAA	0	0	1
DAAAAAAABAABAAA	0	0	1
DAAAAAA/CAAA/CAAAAA	1	0	0
DAAAAABAAAAAAAA	0	0	1
DAAAAABAAAAA/CAAB	1	0	0
	15	40	42

Table 3. Selected chi-square comparisons for American shad in baseline and intercept populations. Abbreviations are as follows: R-Rudee, C-Chincoteague, Q-Quinby, SF-Susquehanna Flats, CHO-Chowan, SAV-Savannah, SAN-Santee, STJ-St. Johns, 90-Susquehanna at Conowingo Dam in 1990, 91-Susquehanna at Conowingo Dam in 1991.

Comparison	Chi-square data	Chi-square simul.	P	Number of hap	Number of indiv
All populations	4765.41		0.000	164	494
R-C-Q	94.78	92.39	0.000	34	97
C-Q	19.45	33.78	0.717	22	82
East coast pop.	2445.18	1349.23	0.000	124	361
Ches. Bay-North	814.74	411.67	0.000	73	219
Chesapeake Bay	169.13	200.76	0.095	52	361
North of Ches. Bay	101.39	135.60	0.088	29	125
South of Ches. Bay	416.02	310.50	0.000	62	142
90-91-SF	213.00	243.04	0.007	97	166
90-91	77.38	112.81	0.467	99	155
90-SF	59.10	103.00	0.858	83	110
90-SAN	80.44	82.42	0.003	89	140
91-SF-SAN	77.52	99.86	0.063	31	108
91-SF	22.10	32.77	0.036	24	67
SF-SAN	17.49	36.71	0.376	17	52
SF-SAV	39.11	49.66	0.039	29	56
SF-STJ	24.19	32.42	0.055	36	39
SF-CHO	15.27	21.83	0.063	11	31

Table 4. Trials and accuracy testing of GIRLSEM to determine ability of the algorithm to estimate stock composition of a mixed sample from mtDNA data. Mixture populations of known composition were created by sampling twelve baseline populations with replacement.

Mixture 1.
25% of all baseline populations sampled with replacement.
36 haplotypes compared.

Source	N	Contribution			Ho:A=E	alpha=
		Actual	Estim.	SE	z	0.001
	96					
1. COL		0.066	0.067	0.036	0.016	accept
2. CT		0.066	0.000	0.025	-2.604	accept
3. DEL		0.104	0.359	0.058	4.405	reject
4. HUD		0.142	0.149	0.051	0.156	accept
5. SF		0.028	0.000	0.017	-1.672	accept
6. NAN		0.066	0.074	0.037	0.222	accept
7. PAM		0.076	0.066	0.037	-0.243	accept
8. JAM		0.113	0.059	0.040	-1.353	accept
9. CHO		0.047	0.000	0.022	-2.181	accept
10. SAN		0.113	0.082	0.043	-0.718	accept
11. SAV		0.094	0.074	0.040	-0.500	accept
12. STJ		0.085	0.068	0.038	-0.428	accept

Mixture 2.
50% of all baseline populations sampled with replacement.
40 haplotypes compared

Source	N	Contribution			Ho:A=E	alpha=
		Actual	Estim.	SE	z	0.0001
	164					
1. COL		0.102	0.097	0.033	-0.152	accept
2. CT		0.059	0.000	0.018	-3.211	reject
3. DEL		0.122	0.304	0.044	4.124	reject
4. HUD		0.108	0.142	0.036	0.931	accept
5. SF		0.031	0.093	0.026	2.381	accept
6. NAN		0.064	0.032	0.024	-1.355	accept
7. PAM		0.085	0.043	0.027	-1.586	accept
8. JAM		0.122	0.047	0.030	-2.468	accept
9. CHO		0.048	0.000	0.017	-2.879	accept
10. SAN		0.096	0.075	0.031	-0.687	accept
11. SAV		0.108	0.111	0.034	0.102	accept
12. STJ		0.055	0.057	0.025	0.086	accept

Mixture 3.
75% of all baseline populations sampled with replacement.
72 haplotypes compared.

Source	N	Contribution			Ho:A=E	alpha=
		Actual	Estim.	SE	z	0.001
	258					
1. COL		0.095	0.091	0.042	-0.095	accept
2. CT		0.060	0.000	0.024	-2.478	accept
3. DEL		0.146	0.295	0.059	2.537	accept
4. HUD		0.122	0.107	0.046	-0.330	accept
5. SF		0.027	0.033	0.025	0.224	accept
6. NAN		0.061	0.061	0.035	-0.002	accept
7. PAM		0.058	0.075	0.036	0.464	accept
8. JAM		0.109	0.081	0.042	-0.658	accept
9. CHO		0.031	0.000	0.018	-1.738	accept
10. SAN		0.126	0.113	0.047	-0.271	accept
11. SAV		0.103	0.092	0.043	-0.252	accept
12. STJ		0.062	0.052	0.033	-0.296	accept

Table 5. Estimated stock composition of groups of American shad.

A. That portion of Virginia's ocean harvest which shared haplotypes with the baseline populations.

B. That portion of Susquehanna River shad lifted over Conowingo Dam in 1990-91 which shared haplotypes with the baseline populations.

C. That portion of Susquehanna Flats shad which shared haplotypes with the baseline populations.

D. That portion of Rudee Inlet, Chincoteague, and Quinby landed shad which shared haplotypes with the baseline populations.

A. Estimated compositon of 81% of Virginia's 1991 intercept fishery. Nineteen percent of intercept shad could not be characterized by the current baseline data set.

Source	Contribution	SD
CT	0.0000	0.0000
DEL	0.0000	0.0000
HUD	0.1477	0.0958
SF	0.0000	0.0000
NAN	0.0000	0.0000
PAM	0.0721	0.0636
JAM	0.0118	0.1309
CHO	0.0000	0.0000
SAN	0.0731	0.1422
SAV	0.0000	0.0000
STJ	0.0421	0.0408
SUS	0.6531	0.1189

B. Estimated composition of 41% of Susquehanna River shad stock (1990-91). Fifty-nine percent of Susquehanna shad could not be characterized by the current baseline data set.

Source	Contribution	SD
COL	0.0000	0.0000
CT	0.0001	0.0001
DEL	0.0359	0.6163
HUD	0.0000	0.0000
SF	0.0000	0.0000
NAN	0.3105	0.1314
PAM	0.1008	0.0945
JAM	0.0000	0.0000
CHO	0.0000	0.0000
SAN	0.3640	0.1364
SAV	0.0000	0.0000
STJ	0.1887	0.5097

C. Estimated composition of 91% of Susquehanna Flats shad in 1991. Nine percent of Susquehanna Flats shad were unique and could not be characterized by the current baseline data set.

Source	Contribution	SD
COL	0.0000	0.0000
CT	0.0000	0.0000
DEL	0.0000	0.0000
HUD	0.5294	0.3342
NAN	0.0000	0.0000
PAM	0.0000	0.0000
JAM	0.0000	0.0000
CHO	0.0206	0.3800
SAN	0.4500	0.2843
SAV	0.0000	0.0000
STJ	0.0000	0.0000

D. Estimated composition of intercept harvests landed at Rudee Inlet, Chincoteague, and Quinby Virginia in 1991 (83%, 93%, and 91% of total samples, respectively). Unique individuals from each location could not be characterized by the current data set.

	Rudee		Chincoteague		Quinby	
Source	Contrib.	SD	Contrib.	SD	Contrib.	SD
CT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
DEL	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
HUD	0.0000	0.0000	0.1740	0.1624	0.1516	0.1414
SF	0.0000	0.0000	0.0000	0.0000	0.0009	0.0022
NAN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PAM	0.6601	0.5244	0.0000	0.0000	0.0000	0.0000
JAM	0.0468	1.6115	0.0000	0.0000	0.0000	0.0000
CHO	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SAN	0.0000	0.0000	0.3544	0.2568	0.0640	0.1541
SAV	0.0979	1.2387	0.0077	0.2004	0.0011	0.0040
STJ	0.0000	0.0000	0.1047	0.0999	0.0000	0.0000
90	0.0000	0.0000	0.3006	0.1718	0.7824	0.1013
91	0.1952	0.1689	0.0585	0.0564	0.0000	0.0000

Appendix A. Genotypes of American shad taken in the coastal intercept fishery outside the mouth of Chesapeake Bay during the Spring of 1991. Enzyme titles are abbreviated versions of those listed in the text on p.7.

		Enzyme Genotype														
Location	ID	Aat	Apa	Bcl	Bgl	Dra	Ecl	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Rudee Inlet	1	D	A	A	A	A	A	B	A	A	A	A	A/C	A	A	B
	2	D	A	A	A	A	A	A/C	A	A	A/C	A	A	A	A	A
	3	-	B	A	A	A	A	A	A	A	B	A	A	-	-	A
	4	-	A	A	-	A	A	A	A	-	A	A	-	-	-	-
	5	A	A	A	F	A	A	A	A	A	A	A	A	A	A	A
	6	-	B	-	A	-	A	-	-	A	B	-	B	A	A	B
	7	-	A	-	-	-	A	-	A	A	-	A	-	-	-	A
	8	-	-	-	-	A	A	A	-	A	A	A	-	-	-	A
	9	-	A	-	A	-	A	-	-	A	-	-	-	-	-	A
	10	-	-	A	-	-	A	-	-	A	-	A	A	-	-	-
	11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	12	-	A	A	A	-	A	-	A	A	A	A	A	-	-	-
	13	-	-	-	-	-	A	-	A	A	A	A	A	-	-	-
	14	A	-	A	-	A	A	-	A	A	A	A	A	-	-	A
	15	A	-	A	A	A	A	A	A	-	A	A	-	-	A	A
	16	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	17	-	A	A	A	-	A	-	A	A	A	A	A	-	A	A
	18	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	19	-	A	A	-	-	A	-	-	A	A	A	A	-	A	A
	20	-	-	-	-	-	A	-	-	A	A	A	-	-	-	-
	21	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
	22	-	A	A	A	-	A	-	A	A	A	A	A	-	A	A
	23	-	-	-	A	-	A	-	A	A	-	-	-	-	-	A
	24	-	A	-	A	-	B	-	A	A	-	-	-	-	-	A
	25	A	A	A	A	A	A	C	A	A	A	A	C	A	A	A
	26	A	A	-	A	A	-	-	-	A	-	-	-	-	-	A
	27	A	-	-	A	A	A	-	-	A	A	A	-	-	-	-
	28	A	-	-	A	A	A	-	A	A	-	-	-	-	-	A
	29	A	A	A	A	A	A	C	A	A	A	A	A/C	A	A	B
	30	A	-	-	A	A	A	-	-	A	-	-	-	-	-	A
	31	D	-	A	A	A	A	-	-	A	A	-	A	-	-	A
	32	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A
	33	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-
	34	A	A	A	A	A	A	C	A	B	A	A	A	A	A	A
	35	A	-	-	A	A	A	-	-	A	-	-	-	-	-	A
	36	A	A	A	A	A	-	C	A	B	A	A	A/C	-	A	B
	37	A	A	A	A	A	A	-	-	A	A	A	A	-	-	A
	38	A	-	-	A	A	-	-	A	A	A	A	-	-	-	A
	39	-	A	-	A	A	A	-	A	B	A	A	A	-	A	-
	40	A	A	A	A	A	A	C	A	B	A	A	A	A	A	B
	41	A	A	A	A	A	A	A	A	B	E	A	A	A	A	A
	42	A	A	A	A	A	-	A	A	B	A	A	A	-	A	B
	43	-	A	A	A	A	-	A	A	B	A	A	B	-	A	-
	44	-	A	A	A	A	A/B	A	A	B	A	A	A	-	A	-
	45	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B
	46	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-
	47	-	A	A	A	-	-	A	A	B	-	A	A	-	-	-
	48	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-
	49	A	-	A	A	A	-	A	-	A	A	-	-	-	A	-
	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	52	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Appendix A. Continued

Location	ID	Enzyme Genotype														
		Aat	Apa	Bcl	Bgl	Dra	EcI	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Chincoteague	1	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
	2	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	4	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	5	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A
	6	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	7	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	8	-	-	A	A	A	A	A	A	-	A	A	A	A	A	-
	9	-	-	A	A	A	A	A	A	-	A	A	A	A	A	-
	10	-	A	A	A	A	A	A	A	-	A	-	B	-	A	-
	11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/B
	13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	14	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
	16	-	-	A	A	A	A	A	A	-	A	A	A	-	A	-
	17	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	18	-	A	A	A	A	A	A	A	A	A	-	-	-	A	-
	19	-	A	A	A	A	A	A	A	A	A	-	-	A	A	-
	20	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	21	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	22	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	23	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	24	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	26	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	27	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	28	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
	29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	30	A	A	A	A	A	-	-	-	A	-	A	A	A	A	-
	31	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-
	32	-	A	A	-	-	-	-	-	-	-	A	A	-	A	-
	33	-	A	A	-	-	-	-	-	-	-	A	B	-	A	-
	34	A	G	A	A	A	A	A	A	A	A	A	B	A	A	A
	35	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A
	36	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	37	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	38	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	39	-	-	-	-	-	-	-	-	A	-	A	A	-	-	-
	40	-	-	A	-	-	-	-	-	-	-	A	A	-	A	-
	41	A	A	A	A	A	-	-	-	B	A	A	B	A	A	A
	42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	43	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	44	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	45	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	47	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	48	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	49	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	50	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	51	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	52	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	53	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	54	-	A	A	A	A	-	A	A	-	A	-	A	A	A	-

Appendix A. Continued

		Enzyme Genotype														
Location	ID	Aat	Apa	Bcl	Bgl	Dra	EcI	EcV	Hin	Kpn	Pst	Pvu	Sal	Sma	Sst	Xba
Quinby	1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	2	-	A	-	-	-	-	-	-	A	-	A	A	-	-	-
	3	-	A	A	A	-	-	-	A	A	A	A	A	A	A	A
	4	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	6	-	-	-	-	-	-	-	A	B	A	A	A	A	A	-
	7	A	A	A	A	A	A	B	A	B	A	A	A	A	A	A
	8	A	A	-	-	-	-	-	A	A	A	-	A	A	A	A
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	A	A	A	A	A	A	A	A	A/B	A	A	A/B	A	A	A
	11	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	12	A	A	A	A	A	A	B	A	B	A	A	B	A	A	A
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
	15	-	A	-	-	-	A	-	-	-	-	-	A	-	-	A
	16	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-
	17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	18	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	19	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	20	-	A	-	-	-	-	-	A	A	A	A	A	A	A	A
	21	-	A	A	A	A	-	-	-	A	A	A	A	-	-	A
	22	A	A	A	A	A	-	A	A	C	A	A	A	A	A	A
	23	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	24	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	25	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	26	D	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	27	D	A	A	A	A	A	B	A	A	A	A	A	A	A	A
	28	D	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	29	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	30	D	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	31	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	32	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	33	A	A	A	A	A	-	A	A	A	A	A	A	-	A	-
	34	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	35	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	36	-	-	-	A	A	-	-	A	-	A	A	A	A	A	A
	37	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	38	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	39	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	40	A	-	A	A	-	A	A	-	-	A	A	A	-	A	-
	41	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	43	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
	44	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	45	B	A	B	A	A	A	A	A	E	A	A	A	A	A	A
	46	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	47	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A
	48	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	49	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	50	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
	51	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	52	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
	53	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	54	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	55	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	56	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Appendix B. Haplotypes of American shad harvested in Virginia's coastal intercept fishery and of shad from fourteen baseline rivers. Abbreviations are as follows: R-Rudee Inlet, C-Chincoteague, Q-Quinby, 90-Susquehanna River at Conowingo Dam in 1990, 91-Susquehanna River at Conowingo Dam in 1991, COL-Columbia, CT-Connecticut, DEL-Delaware, HUD-Hudson, SF-Susquehanna Flats, NAN-Nanticoke, PAM-Pamunkey, JAM-James, CHO-Chowan, SAN-Santee, SAV-SAvannah, STJ-St. Johns.

Haplotypes	Population																
	R	C	Q	90	91	COL	CT	DEL	HUD	SF	NAN	PAM	JAM	CHO	SAN	SAV	STJ
1 AAAAAAAAAAAAAAA	2	16	14	17			15	22	14	6	3	5	5	9	14	3	6
2 AAAAAAAAAAAAAAB		1			1												
3 AAAAAAAAAAAAAAA/B		1															
4 AAAAAAAAAAAAAAD															3		
5 AAAAAAAAAAAAABA				3				1			1						
6 AAAAAAAAAAAAABA/B				1													
7 AAAAAAAAAAAAABD											1						
8 AAAAAAAAAAAABAA											1		1			1	3
9 AAAAAAAAAAAABBA													3			9	
10 AAAAAAAAAAAACAA																1	
11 AAAAAAAAAAABAAA		1	1	2				2			1			1	4		
12 AAAAAAAAAAABABA					1												
13 AAAAAAAAAAACBBA																1	
14 AAAAAAAAAAADAAA																1	
15 AAAAAAAAAABAAAA		1	1					1	4								
16 AAAAAAAAAABAABA													1				
17 AAAAAAAAAABABBA																1	
18 AAAAAAAAAA/BAAAAA				1													
19 AAAAAAAAAA/CAAAAA				1													
20 AAAAAAAAAA/CABAAA/B				1													
21 AAAAAAAAACAAAAA								1									
22 AAAAAAAAACAAAAA													8				1
23 AAAAAAAAADAAAAA							1										
24 AAAAAAAAAEAAAAA																	1
25 AAAAAAAAAFAAAAA															1		
26 AAAAAAAAA/BAAA/BAAA			1														
27 AAAAAAAABAAAAAA	1	4	5	5			6	8	2			1	3		2	2	2
28 AAAAAAAABAAAAAB	1											2	1	2			
29 AAAAAAAABAAAABA	1				3												
30 AAAAAAAABAAABBA								1								6	
31 AAAAAAAABAAACBA																1	
32 AAAAAAAABAABAAA	1	7	3		2		2	2	5	2		2	2		7	1	
33 AAAAAAAABAABABA					1						1						
34 AAAAAAAABAABACA															1		
35 AAAAAAAABAABBAA																	1
36 AAAAAAAABAABBBA								1								1	
37 AAAAAAAABCAAAAA													1				
38 AAAAAAAABCAABBA													1				
39 AAAAAAAABCBBAAA													1				
40 AAAAAAAABC/AABAAA													1				
41 AAAAAAAABFAABBA																1	
42 AAAAAAAACAABAAA													1				
43 AAAAAAAACAABBBA																1	
44 AAAAAAAADAAAAAA								1									
45 AAAAAAAADAAAABA											1						
46 AAAAAAAAECAAAAA				1													
47 AAAAAAABAAAAABA									1								
48 AAAAAAABAAAABBA																1	
49 AAAAAAABEAAAAAA	1			1													
50 AAAAAAACAAAAAAA			1						1								
51 AAAAAAA/CAAAAABAA/B				1													
52 AAAAAAA/CABAAAAAA				1													
53 AAAAAAA/CABEAAAAA				1													
54 AAAAAABAAAAAAAA				3					4		3	1					
55 AAAAAABAAAABAAB											1						
56 AAAAAABAAACAAAA									2								
57 AAAAAABAAA/CAAABA				1													

		Population														
Haplotypes	R	C	Q	90	91	COL	CT	DEL	HUD	SF	NAN	PAM	JAM	CHO	SAN	SAV	STJ
58 AAAAAABABAAAAAA			1	1													
59 AAAAAABABAABAAA			1														
60 AAAAAACAAAAAAAA				4				1		2	4	3					
61 AAAAAACAAAAAAAA/B				1													
62 AAAAAACAAAAAAAD				1													
63 AAAAAACAAAAABBA											1						
64 AAAAAACAAAABAAA												1					
65 AAAAAACAAAACAAA	1																
66 AAAAAACAAAAA/CAAB	1																
67 AAAAAACABAAAAAA	1											2					
68 AAAAAACABAAAAAB	1																
69 AAAAAACABAAAABA												1					
70 AAAAAACABAABAAA												2					
71 AAAAAACABAABAAA												1					
72 AAAAAACACAABAAA												1					
73 AAAAAACABAAA/CAAB	1																
74 AAAAAACBAAAAAAA													1				
75 AAAAAACBAAAAABD				1													
76 AAAAAACBAAAABBA											1		1				
77 AAAAAACBAAACBBA											1						
78 AAAAAACBACAAABA													1				
79 AAAAAACBBAAAABA																1	
80 AAAAAACBBAAC/BBBA											1						
81 AAAAAACBDAAAAAA											1						
82 AAAAAACCAAAAAAA													1				
83 AAAAAACCAAAAABA				1													
84 AAAAAACCAAAABBA											1						
85 AAAAAACCCAACBBA											1						
86 AAAAAADBBAAAABA				1													
87 AAAAABAAAAAAAAA		1															2
88 AAAAABAAAA/CAAAAA				1													
89 AAAAABAABAAAAAA															1		
90 AAAABAAAAAAAAAA		1															
91 AAAABBAAAAAAAAA													1				
92 AAAA/EAAAAAAAAAAA						26			2								
93 AAAA/EAAAABAAAAAA									3								
94 AAAA/EAABAAAAAAAA						3											
95 AAAA/EAACAAAAAAAA						5											
96 AAAAA/EACAAAAAAAA				1													
97 AAABAAAAAAAAAAA												1					
98 AAABAAAAAAAABAA								1									
99 AAABAACAAAAAAAA												1					
100 AAACAAAAAAABBBA																1	
101 AAACAAAABAAABBA																1	
102 AAAEAAAAAAAAAAA						1											
103 AAAEAACCABAAAAA						1											
104 AAAFAAAAAAAAAAA	1																
105 AABAAAAABAAAAAA								1							1		
106 AACAAAAAAAAAAAA								1									
107 AACAAAAAAABAAAA									1								
108 AADAAAAAAAAAAAA															2		
109 AADAAAAABAAAABA				1													
110 AADAAAAAAAAABBA													1				
111 AADAAAAACCABAAC													1				
112 AAEAAAAAAAAAAAA															1	1	
113 AAEAAAAAACABBAA																1	
114 ABAAAAAAAAAAAAA		1															

Haplotypes	Population																
	R	C	Q	90	91	COL	CT	DEL	HUD	SF	NAN	PAM	JAM	CHO	SAN	SAV	STJ
115 ABAAAAAAAAAABAA								2									
116 ABAAAAAAAAAABAA																	3
117 ABAAAAAAAAACAAA								1									
118 ABAAAAAAACAAAAA													2				
119 ABAAAAAABBAAABA					1												
120 ABAAAACAAABAAAA												1					
121 ABAAAACABBAAAAA												1					
122 ABAAABAAAAAABAA																	1
123 ABACAAAAACAAAAA								1									
124 ACAAAAAAAAAAAAA																1	3
125 ACAAAAAAAAAABAA																	1
126 ACAAAAAAACAAAAA																2	
127 ACABAAAAAAAACBA																2	
128 ADDAAACBAAAAAAA												1					
129 AEAAAAAAAAAAAAA												1					
130 AEAAAACAAAAAAAA												1					
131 AEAAAACBBBAAAAA												1					
132 AA/EAAAACBAAAABBA					1												
133 AFAAAAAAAA/BABAAA				1													
134 AGAAAAAAAAABAAA		1															
135 BAAAAABAAAAAAAA/B				1													
136 BAAAAACCAAAAAAA				1													
137 BABAAAAAEAAAAAA			1														
138 BBAAAAAAAEAABAA																	1
139 BBAABACABAAAABA				1													
140 CAAAAAAABBABAAA				1													
141 CAAAAAAACAAABBA																1	
142 CAAAAAABBA/CAAABA				1													
143 CAAAAACAAAAAAAA				1								1					
144 CAAAAAA/CAAAAAAAA				2													
145 CAAAAACAAAAAAAA/B				1													
146 CAAAAACBAAAABBA																1	
147 CAAAAACBAAABBBA																1	
148 CAAAAACBAA/CAAAAA				1													
149 CAAAABCBAAAAAAA																	1
150 DAAAAAAAAAAAAAA		5	8	4											1		
151 DAAAAAAAAAAABAA																1	
152 DAAAAAAABAABAAA										1							
153 DAAAAAAAAABAAAA			2	1													
154 DAAAAAAAAA/CAAAAA				1													
155 DAAAAAAAABAAAAA				1													
156 DAAAAAAABAAAAAA			1	1													
157 DAAAAAAABAABAAA			1	1													
158 DAAAAAA/CBAAAAABA				1													
159 DAAAAAA/CAAA/CAAAAA	1																
160 DAAAAABAAAAAAAA			1														
161 DAAAAABAAAAA/CAAB	1																
162 DAAAAACAAAAAAAA				1													
	15	40	42	75	10	36	24	48	39	11	24	31	38	12	38	45	26